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A Study of the Chemistry of Incipient Oxidation Defects in Butter[1]

C. R. BARNICOAT[2] and L. S. PALMER

When butter is kept in cold storage, the flavor alters even though the product is of the finest quality, and the desirable properties of "freshness" and "bloom" gradually disappear. After prolonged storage, a certain degree of staleness may appear even in the finest product, and this is sometimes accompanied by other mild off-flavors, described as "bitter," "metallic," "oily," "oxidized," "storage," etc. At this stage the butter is still quite palatable; in fact these flavors probably could not be detected by the average consumer. However, the price of butter depends on its grade (score), which is based principally on the flavor, and a lack of freshness, as described, detracts from its commercial value.

There is an enormous volume of technical and scientific literature dealing with the subjects of flavor, flavor defects, and keeping quality of butter, which are interrelated. For the purposes of this discussion these may be briefly summarized as follows.

TYPES OF BUTTER

I. Sweet Cream Butter (includes most of the finest butter).
Salted and Unsalted

A. Non-starter butter. Very mild flavor, derived from the natural ingredients (fat, lactose, protein, salts, etc.), but modified apparently by traces of substances of unknown composition, derived originally from the feed of the cows.

B. Starter butter.

1. Mild starter butter, from cream containing 1 per cent or less of starter culture, unripened, giving butter of low acidity and mild flavor.
2. Starter butter, made from cream containing a considerable proportion of starter culture (5 to 15 per cent), usually subjected to a ripening process. The flavor is derived mainly from traces of substances such as diacetyl, esters and acids, and carbonyl compounds produced by the growth of the starter organisms (*Streptococcus* and associated types) in the cream prior to churning.

[1] A thesis presented to the faculty of the Graduate School of the University of Minnesota by C. R. Barnicoat in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1938.

[2] Commonwealth Fund Service Fellow, University of Minnesota, 1936-1938, on leave from Department of Scientific and Industrial Research, Government of New Zealand.

II. Sour Cream Butter (includes centralizer butter). Most of the butter manufactured in the United States is made from sour cream which has been neutralized prior to pasteurization.

Salted and Unsalted

A. Non-starter butter. Usually rather "coarse" and "old cream" flavor, sometimes "cheesey" and otherwise unattractive, which suggests undesirable changes in the protein due to fermentation of the cream.

B. Starter butter. Starter culture is usually added in large amounts, or the cream is ripened considerably, in order to develop a desirable flavor which will mask other undesirable flavors present in the poor-quality cream.

Butter made from sweet cream is invariably of better quality than that made from old, sour cream, and usually has superior keeping properties. The most common defects which are summarized below are also related to the type of butter.

DEFECTS IN FLAVOR OF BUTTER AFTER STORAGE

I. Defects caused by microbiological contamination (more commonly encountered in unsalted or lightly salted butters).

A. "Surface taint" (bacterial).

B. Surface mold.

C. Various flavor defects. Hammer (1928) considers that deterioration due to the presence of microorganisms in butter is unlikely under modern commercial storage conditions.

Defects of these types are usually eliminated by means of careful attention to details of pasteurization and plant hygiene.

II. Defects caused by enzyme action. Rancidity and bitterness caused by the action of lipase (Palmer, 1922). Efficient pasteurization of the cream soon after collection controls this defect, which is uncommon.

III. Defects caused by errors in manipulation.

A. Faulty pasteurization, e.g. too high temperatures giving caramelized or even scorched flavors.

B. Overworking (particularly salted butter) may promote "fishiness" (Sommer and Smit, 1923).

C. Oversalting also tends to cause "fishiness."

D. Exposure of cream and butter to excess light (particularly sunlight) may cause "oily" or "tallowy" flavors, often accompanied by bleaching of the yellow pigment (carotene) in the fat.

IV. Defects caused by metallic contamination. Traces of metals (usually copper and iron) tend to give metallic, "oily," "tallowy," and "fishy" flavored butter.

V. Defects caused by excessive acidity. In unsalted butters these may appear as "coarse" or "vinegary" flavors; in salted butters the development of "tallowy" and "fishy" flavors is accelerated.

VI. Defects caused by feed flavors which can sometimes be eliminated by careful attention to feeding methods and by "deodorization" (vacuum treatment) in the creamery.

Prolonged storage of butter, particularly at relatively high temperatures, encourages the development of off-flavors.

The factors promoting the different types of deterioration are related, and it is therefore not surprising to find that the flavor defects are usually of a complex nature. For example, a fishy butter will be appreciably stale and probably somewhat oily, or even tallowy. Nevertheless, experienced butter graders can usually give accurate opinions on the nature of flavor defects, and usually agree closely with one another with regard to the numerical score (market grade) of the samples. This point is stressed, because much of the work presented in this study depends entirely on the results of grading by flavor of over two hundred samples of experimental butter manufactured in the course of this investigation.

Many of the defects previously described, which formerly caused a considerable financial loss to the butter trade, have now been eliminated by means of careful attention to such details as plant hygiene, efficient equipment (particularly pasteurizers and cooling units), replacement of badly tinned copper equipment and monel metal vats by stainless steel, and protecting the cream and butter against the action of light.

The tendency to develop off-flavors is, as noted, related to the type of butter manufactured and it was also mentioned that most of the butter made in the United States and abroad is made from sour cream, or from cream which has been ripened. The flavor and keeping properties of these products are therefore determined, it is believed, mainly by biological processes.

On the other hand, an increasing proportion of the finest butter is made from sweet cream which has undergone a negligible amount of bacterial action and is unripened. In order to manufacture this product successfully, the cream must be very carefully treated, held at low temperatures, and delivered to the creamery soon after milking. Much of the butter made in Minnesota (and most of that made in New Zealand for the markets of the United Kingdom) is of the sweet cream type. Owing to its initial fine quality, it has excellent keeping properties and is frequently held in storage at −5°F. to about 15°F. for many months before consumption. Pronounced defects such as tallowy or fishy flavors are rare, but a certain amount of "storage staleness," often accompanied by other incipient off-flavors appears after a long period of storage.

Although there is an extensive literature dealing with flavor defects in butter, the incipient off-flavors have received little attention. Sweet-cream butter with incipient off-flavors is still superior to the majority

of butters of the sour-cream type when freshly made. Research has mainly been directed toward improving the poorer product.

The nature of incipient flavor defects in sweet cream butter can be explained only by conjecture. As they are found frequently in salted butter, which has been held at 40°F. and lower since manufacture, this may be taken as presumptive evidence that they are not of microbiological origin. The fact that sweet cream butters vary in their susceptibilities toward storage flavors is of interest, as it suggests variations in composition, or treatment of the product, as being factors concerned in determining keeping quality.

Work carried out by one of the authors in New Zealand more than two years ago gave indications that an oxidation process was concerned in the development of storage staleness, and the initial part of the present investigation, recorded mainly in Part I of this bulletin, deals with experiments made in order to test this theory.

Part II deals with experiments designed to trace the substances responsible for certain incipient off-flavors.

Part III considers the rôle of various substances in butter in relation to the development of incipient oxidation defects.

All of the work presented in this bulletin must be regarded as being of an exploratory nature. The conditions of manufacture for the experimental butters were standardized as closely as possible to normal factory procedure in the State of Minnesota, which differs only in details from that used elsewhere in this country. The cream was from the University Farm herd. The conditions of cold storage, though rather more drastic than average commercial practice, were constant. No full-scale creamery trials have been made, and the results must therefore be regarded as being of qualitative significance only.

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I. Experiments on the Oxidation of Butter Constituents

As fat is the major constituent of butter, approximately 80 per cent, and as it is well known that oxidation is the most important cause of deterioration in other edible fats and oils, it is usually assumed that incipient oxidation of the fat is responsible for flavor defects in butter which cannot be ascribed to the action of microorganisms, or to other specific causes. Butter normally contains from 4 to 6 per cent by volume of air.

An interesting paper by Dyer (1916) which seems to have been overlooked by present-day workers presented much indirect evidence to the effect that the oxidation of non-fatty substances was also of considerable importance in the process of butter deterioration. Dyer concluded

that the unpleasant flavors which develop in butter during cold storage are not produced by oxidation of the fat itself but by some chemical change which takes place in one or more of the non-fatty ingredients.

Within recent years, a considerable amount of evidence has also been presented which indicates that certain incipient off-flavors in market milk, similar to those produced by the catalytic action of light, and/or metals, and therefore termed "oxidized," are caused by oxidation, not of the fat, but of the plasma ingredients, probably the phospholipides (Thurston, Brown, and Dustman, 1935, 1936; Dahle and Palmer, 1937). These findings are in harmony with those of Dyer.

Experiments were undertaken in order to test Dyer's hypothesis.

EXPERIMENTS ON THE OXIDATION OF THE NON-FAT CONSTITUENTS OF BUTTER (BUTTERMILK)

EXPERIMENT 1: To 100-ml. portions of buttermilk from sweet, unripened, pasteurized cream, containing 10 grams of salt (with 10 per cent sodium fluoride as preservative, and 1 ml. of toluene), the following amounts of metal were added:

a. 0.25 ppm Cu″ *b.* 5 ppm Cu″ *c.* 2 ppm Fe″ *d.* 10 ppm Fe″ *e.* nil—control.

These buttermilks were held in glass-stoppered bottles in the dark at 70-75°F., and were aerated by shaking at intervals.

5-ml. portions were withdrawn and titrated by Foreman's method (1920). The results are given in Table 1.

The total basicity toward phenolphthalein ("total acidity") remains practically unchanged. There is a slight increase in free amino nitrogen.

EXPERIMENT 2: To buttermilk containing 10 per cent NaCl: NaF mixture, the following amounts of metal were added:

a. 0.25 ppm Cu″ *b.* 5 ppm Cu″ *c.* 10 ppm Fe″ *d.* nil—control.

Table 1. Oxidation of Buttermilk by Air, in the Presence of Traces of Iron and Copper

(Results expressed in ml. $\frac{N}{20}$ NaOH/5 ml. buttermilk)

	Direct titration (free acidity)	+Neutral alcohol (internal salt acidity)	Total acidity	+HCHO (amino nitrogen)
Initial	2.15	0.50	2.65	1.60
After 12 days				
a.	0.95	1.90	2.85	Nil
b.	0.95	2.00	2.95	Nil
c.	0.85	2.00	2.85	Nil
d.	0.90	1.85	2.75	Nil
e.	0.80	1.80	2.60	Nil
After 26 days				
a.	0.90	1.90	2.80	0.60
b.	1.15	1.60	2.75	0.50
c.	1.00	1.65	2.65	0.40
d.	0.95	1.80	2.75	0.40
e.	0.95	1.80	2.75	0.55

As an oxidant, perhydrol was added, so that the solutions originally contained 1 per cent H_2O_2.

5-ml. samples were withdrawn, a pinch of a catalase preparation (acetone-treated yeast) was added to each, and the titrations were made by Foreman's method. The results are given in Table 2.

Table 2. Oxidation of Buttermilk by Hydrogen Peroxide, in the Presence of Traces of Metals (Results expressed in ml. $\frac{N}{20}$ NaOH/5 ml. buttermilk)

	Direct titration (free acidity)	+Neutral alcohol (internal salt acidity)	Total acidity	+HCHO (amino nitrogen)
After 2 days				
a.	1.05	1.70	2.75	0.15
b.	2.55	1.30	2.85	0.20
c.	0.95	1.60	2.55	0.15
d.	0.95	1.55	2.50	0.25
After 8 days				
a.	1.65	1.45	3.10	0.15
b.	5.80	0.95	6.75	0.15
c.	1.45	1.40	2.85	0.05
d.	1.35	1.60	2.95	0.15
After 28 days				
a.	2.45	Nil	2.45	1.05
b.	7.95	0.15	8.10	0.85
c.	1.80	0.90	2.70	0.70
d.	2.20	0.50	2.70	0.60

With the exception of *b,* which contains a very high amount of copper, the total basicity toward phenolphthalein ("total acidity") is practically unchanged. There is a slight increase in the free amino nitrogen.

These results indicate that even in the presence of relatively large amounts of copper and iron, and under very favorable conditions for oxidation, the ingredients of buttermilk are apparently rather stable.

EXPERIMENT 3: As aldehydes are believed to be mainly responsible for the typical flavor of an oxidized fat, 10 ml. portions of each of the samples from series 2 were distilled, after adding "catalase," and the distillates tested for aldehydes with p-nitrophenyl hydrazine with the following results:

Series 2. *a.* trace *b.* considerable—probably weighable *c.* trace *d.* trace. Lactose gave no precipitate.

A further study was not made because it was foreseen that this method would not be applicable to commercial butter, because of the possible interference by volatile carbonyl compounds arising from bacterial action. The conditions of oxidation were, in any case, much more drastic than might be expected in commercial butter.

It was possible that the oxidation of the proteins might not have proceeded so far as an actual degradation, and the only tests which could be found which would detect initial stages were those of Gortner and Holm (1920) who used the Folin and Denis (1912) reagent, and simple

tests for dioxyphenylalanine (dopa) (Abderhalden, 1930), and the well known indole test (Ehrlich's). None of these tests is by any means specific for protein oxidation, however.

EXPERIMENT 4: Two milliliters of buttermilk, obtained from the samples described under 1 and 2 were tested with the Folin and Denis and the ferric chloride reagents. The results are given in Table 3.

The ferric chloride test is more specific for dioxyphenylalanine than the other. Hydrogen peroxide in the presence of 5 ppm Cu" (2) is a relatively powerful promotor of a reaction for oxidized protein, but naturally soured buttermilk, in which biological action is the predominating effect, gives an equally strong reaction.

Table 3. Tests for Oxidized Proteins in Buttermilks Containing Traces of Metals, etc.

Sample	Held at 70-75°F. for—	Folin and Denis reagent	Ferric chloride test
Series 1			
a-e.	11 days	Very faint positive reactions Control e least	Much less than 0.1 mgm. dopa/2 ml.
a-e.	40 days	All equal to approximately 0.025 mgm. dopa	.....
Series 2			
a.	11 days	Faint reaction—more than series 1	Much less than 0.1 mgm. dopa/2 ml.
b.	11 days	Strongest reaction—less than 0.25 mgm. dopa/2 ml.	Much less than 0.1 mgm. dopa/2 ml.
c.	11 days	Faint reaction	Much less than 0.1 mgm. dopa/2 ml.
d.	11 days	Faintest reaction	Much less than 0.1 mgm. dopa/2 ml.
		Reaction equal to:	
a.	40 days	0.10 mgm. dopa/2 ml.	.....
b.	40 days	0.15 mgm. dopa/2 ml.	.....
c.	40 days	0.07 mgm. dopa/2 ml.	.....
d.	40 days	0.07 mgm. dopa/2 ml.	.....
Stale, sour buttermilk	.....	0.15 mgm. dopa/2 ml.	.....
Fresh milk	1 day	0.02 mgm. dopa/2 ml.	.....

EXPERIMENT 5: Buttermilks, obtained by carefully melting butters, were next examined in 1-ml. portions. The results are given in Table 4.

Apparently the proteins are not concerned in the deterioration of butter by oxidation, for there is no correlation between the nature of the sample examined and their response to these tests.

The presence of substances giving a reaction for indole is, of course, usually regarded as evidence of bacterial action (protein decomposition) in the cream prior to churning. Clarke and others (1937) have found many anomalies in attempting to make this test quantitative.

Attempts were made next to find evidence concerning the degradation of the phospholipides. As noted previously, certain workers believe that

Table 4. Tests for Oxidized Proteins in Buttermilks Obtained from Butter

Sample	Folin and Denis reagent=mgm. dopa	$FeCl_3$ test	Indole test
45 days' storage at 70-75°F.			
Butter containing:			
1 ppm Cu"	0.04	Nil	Trace
10 ppm Fe"	0.06	Nil	Trace
Butter held in diffuse light	0.05	Nil	Trace
Butter held in the dark			
a.	0.04	Nil	Trace
b.	0.05	Nil	Most—possibly 0.02 mgm. indole
Several months' cold storage			
Commercial (off-flavored) butters:			
Stale storage	0.05	Nil	Trace?
Slightly tallowy	0.06	Nil	Trace?
Fishy			
a.	0.07	Nil	Trace?
b. Reworked	0.06	Nil	Trace?

oxidation of the lecithin, or lecithoprotein, fractions of milk is responsible for incipient "oxidized flavors" in market milk.

Little is known concerning the chemistry of the degradation of phospholipides. Apparently lecithin is hydrolyzed and oxidized, eventually to choline and possibly to trimethylamine which, it is believed, causes fishy flavors in butter.

EXPERIMENT 6: It was felt that the presence of free choline would be evidence of degradation of the phospholipides in butter, and a considerable amount of time was spent comparing methods for its estimation. The methods tried included: 1. Precipitation with Reinecke's acid (Kapfhammer and Bischoff, 1930) after removing interfering substances with ammonium sulphate, acetic acid, trichloracetic acid, or methyl alcohol. This reaction is specific for secondary and tertiary amino groups only. 2. Precipitation of the periodide with Stanek's reagent (Booth, 1935) after removing the proteins with lead acetate. 3. Precipitation with gold chloride (Dudley, 1929).

Reinecke's acid gave the most promising results with aqueous solutions, a precipitate being given by 0.1 mgm. choline (hydrochloride)/6 ml., in both acid and neutral solutions.

When the various buttermilk samples, described in experiments 1, 2, 4, and 5, were treated with Reinecke's acid, the precipitates were gelatinous rather than crystalline, and there was no correlation between the volume of the precipitate and the nature of the samples. The precipitates increased notably on standing several more days, which may be caused by degradation of the phospholipides.

EXPERIMENT 7: Later in the course of this work (see Part II), butters were churned from washed cream, i.e., cream consisting essentially of fat emulsified in water by lecithoprotein, and synthetic creams made by emulsifying butterfat in water with egg lecithoprotein, gelatin, skim

milk, etc. Comparative tests for oxidized protein were made on various fractions using the method of Gortner and Holm (*loc.cit.*), i.e. the Folin and Denis reagent. The results are given in Table 5.

The results are anomalous. Apparently the important lipide fraction of the lecithoprotein gives no response to the test. Presumably the protein fraction, whether oxidized or not, is responsible for the reaction.

Table 5. Tests for Oxidation in the Lecithoprotein Fraction of Artificial Butters and Various Other Phospholipide-Containing Products

	Sample	Relative strength of reaction
1.	Egg lecithoprotein in 4% NaCl—held seven days at 45°C.	+++++++++
2.	"Buttermilk" from butter made from butterfat emulsified with (1)	++++
3.	"Buttermilk" from butter made from butterfat emulsified with (1)+ 0.25 ppm Fe"	+++++
4.	"Buttermilk" from butter made from butterfat emulsified in 0.5% gelatin	+
5.	"Buttermilk" from washed cream butter	+++
6.	"Buttermilk" from washed cream mixed with skim milk	++
7.	Egg lecithoprotein—0.5% in 4% NaCl (14 days old)	+++
8.	Egg lecithoprotein—0.5% in 4% NaCl one day in sunlight	++++
9.	Very stale, brown and oxidized lecithin dispersed in water	+
10.	Alcohol extract of egg lecithoprotein	+
11.	Alcohol and ether extract of egg lecithoprotein	+++
12.	Buttermilk (cultured)	++++
13.	Fresh pasteurized milk	+++
14.	Fresh egg yolk in 4% NaCl	++++++
15.	Butterfat from stale butter	+
	Blanks—alcohol, water or 4% NaCl solution	Nil

EXPERIMENT 8: A characteristic compound was sought on treatment of oxidized egg lecithoprotein with Reinecke's acid. The results were as follows:

	Precipitate
0.5 grams in 4% NaCl egg lecithoprotein (in 80% CH_3OH, filtered)	+
0.5 grams in 4% NaCl egg lecithoprotein (exposed 1 day to light, then in 80% CH_3OH, filtered)	++++
Blank (4% NaCl, etc. and CH_3OH)	Nil

The precipitates were partly dissolved on dilution, which is not expected of the "Reineckates" of choline and allied substances.

It was concluded that the chemical tests available for detecting possible degradation products of lecithin or lecithoprotein kept under conditions likely to promote oxidation were unsatisfactory. This line of work was accordingly dropped.

As previously mentioned, fat oxidation is usually believed to be responsible for flavor defects in butter which cannot be traced to other causes. The authors, in common with other workers in this field, have never been able to find evidence of more than a trace of oxidation in carefully prepared fat obtained from finest butter after storage. The pos-

sibility that a mere trace of a product formed during the earliest stages of fat oxidation (before any response to chemical tests appears) is responsible for storage flavors was next considered.

For this theory, we must assume that fats in different incipient stages of oxidation, i.e. at different stages of their induction periods, would produce storage flavors in butter in proportion to their states of oxidation. It would follow that by accelerating this potential oxidation, e.g. by heating, the oxidation would eventually reach a stage where it would respond to chemical tests. The relative values of the chemical tests in a series of butterfats would then be related to the incipient oxidation of the fat when sampled.

After preliminary trials, an accelerated oxidation test for butterfat was developed and standardized as follows: Six grams of clear fat, obtained by carefully melting butter (usually at 45°C.) are decanted into a straight-sided glass crystallizing dish, 4.8 cm. in diameter and 3.5 cm. in height. The volume/surface of the fat is therefore constant. The dishes are heated for six hours in an air-stirred water-jacketed oven thermostatically controlled at 80°C. As butter is normally stored at approximately —20°C. the accelerated treatment would correspond to eight-months' storage at the lower temperature, assuming the thermal coefficient for the oxidation rate is 0.2°C. temperature increase.

Numerous tests have been published for detecting oxidation in fats. The fat-aldehyde test of Schibsted (1932) applied to butterfat obtained from commercial butters, particularly from the surfaces, responds to this test whereas other methods fail to give results. Even the sensitive peroxide test (Lea, 1931), which has been of great service for oxidation studies on other edible fats, is, as will be shown later, of little use for butter.

Schibsted considers that the reaction between an oxidized fat and a modified Schiff's aldehyde reagent is given by fat aldehydes, which are soluble in petroleum ether to a red color. The color is compared with a standard but the results are, unfortunately, only empirical.

The fat aldehydes are probably flavorless, but the lower molecular weight, water-soluble aldehydes, which are formed in equivalent amounts upon oxidation of the ethenoid linkages of the fatty acid radicals are believed to give rise to certain oxidized and tallowy flavors.

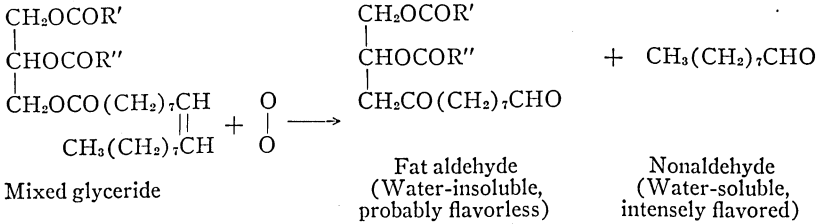

Table 6. Accelerated Test—Six Hours at 80°C.

No.	Results given per ml. fat	Fat-aldehyde value (Schibsted)			Peroxide value (Lea)		
		Unheated	Heated	Increase	Unheated	Heated	Increase
1.	Control butterfat α	0.15	0.7	0.55	0.15	0.45	0.30
2.	Butterfat α +0.1% very oxidized fat	0.2	0.9	0.7	0.25	0.85	0.6
3.	Butterfat α +0.5% very oxidized fat	0.35	1.9	1.55	0.45	0.85	0.4
4.	Butterfat α +1.0% very oxidized fat	0.85	3.25	2.4	0.60	0.85	0.25
5.	Butterfat α +5.0% very oxidized fat	6.7	11.8	5.1	2.20	3.50	1.30
6.	Butterfat α +0.5 ppm active oxygen*	0.15	1.15	1.0	0.20†	0.50	0.30
7.	Butterfat α +1.0 ppm active oxygen	0.15	1.45	1.3	0.25†	0.60	0.35
8.	Butterfat α +2.5 ppm active oxygen	0.15	8.2	8.05	0.30†	2.45	2.15
9.	Butterfat α +5.0 ppm active oxygen	0.15	40.8	40.6	0.45†	8.65	8.20
10.	Butterfat α +0.1 ppm Fe''' (as stearate)	0.15	1.1	0.95	0.15	0.6	0.45
11.	Butterfat α +0.25 ppm Fe''' (as stearate)	0.15	1.25	1.1	0.15	0.6	0.45
12.	Butterfat α +0.5 ppm Fe''' (as stearate)	0.15	1.75	1.6	0.15	0.9	0.75
13.	Butterfat α +1.0 ppm Fe''' (as stearate)	0.15	7.8	7.65	0.15	1.95	1.8

* Added as benzoyl peroxide, which contains 6.6 per cent active oxygen.

† Calculated.

EXPERIMENTS ON THE OXIDATION OF BUTTERFAT

EXPERIMENT 9: The results in Table 6 indicate the reliability of the test. Some peroxide values (Lea) expressed in ml. $\frac{N}{500}$ thiosulphate/ml. fat are also included. In samples 7, 8, 9, and 13 the carotene was bleached, while the others were still quite yellow. Experiments made with another sample of butter are described in Table 7.

Table 7. Accelerated Test—Six Hours at 80°C.

No.	Fat-aldehyde value (Schibsted)		
	Unheated	Heated	Increase
1. Butterfat *b*	0.1	1.0	0.9
2. Butterfat *b* +0.1 ppm Fe‴ (as stearate)	0.1	1.4	1.3
3. Butterfat *b* +0.25 ppm Fe‴ (as stearate)	0.1	2.1	2.0
4. Butterfat *b* +0.5 ppm Fe‴ (as stearate)	0.1	2.45	2.35
5. Butterfat *b* +1.0 ppm Fe‴ (as stearate)	0.1	4.45	4.35
6. Butterfat *b* +0.02 ppm Cu″ (as acetate)	0.1	1.6	1.5
7. Butterfat *b* +0.05 ppm Cu″ (as acetate)	0.1	1.9	1.8
8. Butterfat *b* +0.1 ppm Cu″ (as acetate)	0.1	3.95	3.85
9. Butterfat *b* +0.25 ppm Cu″ (as acetate)	0.1	10.0	9.9

As will be seen in Tables 6 and 7, the presence of small amounts of oxidized butterfat, peroxide as benzoyl peroxide, Fe‴ and Cu″ accelerates the oxidation. The relationship between the fat-aldehyde value and the proportion of oxidant added is not strict. This is partly due to unavoidable errors in technique, and it must also be remembered that a fat undergoing oxidation has a period of induction after which it reacts at a logarithmic rate.

The period of induction appears to end at about two units of fat-aldehyde value. For most of the results obtained with this test the fats have still been in the induction stage, during which the rate of oxidation would appear to be linear.

Other aspects of this test will be discussed when the results of the experiments are considered.

The degree of accuracy expected of an empirical test of this nature is not high, particularly as the presence of the yellow color of the butterfat makes the colorimetric comparisons difficult. The fat-aldehyde values usually obtained (<0.5 unit) actually give rather faint colors, and it is believed that the comparison of these very slightly oxidized fats is rather straining the test beyond the limits of sensitivity considered by its original author. The fat-aldehyde values for butterfat are probably reproducible to only about ± 5 per cent.

An occasional error remained unnoticed until a considerable amount of work had been done. Certain series of fats obtained by decantation from melted butters gave higher fat-aldehyde values than were given by the same butters after further storage. This anomaly was traced to the effect of minute particles of curd, evidently held in the fat: petroleum ether phase in a colloidal suspension which failed to settle out after the

usual two hours' standing. As this trouble was usually encountered in samples examined on the day after manufacture, it would seem that the inversion of the oil/water emulsion (cream) to the water/oil emulsion (butter) had been incomplete, doubtless owing to the difficulty of working the butter satisfactorily by hand.

These particular results, which are shown in brackets, are not valueless, however, as the differences between the test samples and the control, rather than the actual oxidation values, are of most interest.

BUTTERMAKING TECHNIQUE

About two hundred experimental butters were made in small glass Dazey churns. The churns, surrounded by running water at approximately 50°F., were mechanically driven in tandem. Fresh cream obtained from the Station herd, standardized to 35 per cent butterfat, was pasteurized for 30 minutes at 160°F. It was usually pasteurized in glass flasks, but sometimes in well-tinned containers. About 30 minutes was required for heating the cream to the pasteurizing temperature. During the whole of the heating period the cream was stirred with glass rods, care being taken to mix each lot in a similar manner. The creams were cooled overnight to 50°F. and churned at this temperature. The butter granules were washed once, except where otherwise noted, with tap water filtered through a "Seitzwerke" unit. The granules were worked by hand on an aluminum plate with a wooden paddle. No attempt was made to standardize the moisture content. The samples were salted at the rate of 2 per cent, using high-grade dairy salt. The pH of the buttermilk was approximately 6.6. The prints, usually of 2-pound size, were wrapped in parchment and placed in the cooler soon after manufacture.

STORAGE AND GRADING

The samples were held at 40°F. Gradings and oxidation tests were usually made one day, 4½ weeks, and 8½ weeks after manufacture.

SMALL-SCALE LABORATORY CHURNINGS

Many samples, particularly butters made from washed and synthetic creams, were churned by shaking in 8-ounce glass bottles fitted with corks covered with aluminum foil. They were usually washed with distilled water and worked with a flattened glass rod. Salting was also carried out in this way, but the butters were always of rather poor texture.

SCORING

In each series of churnings the cream was divided into several lots, one of which was the control. To the others, various substances suspected of causing oxidation changes in the cream and butter were added.

As the only variables in each series were the substances added, differences in flavor and score between the control sample and the others were ascribed to their action. The butters were examined at intervals by fat-oxidation tests as well as by grading.

The grading tests were made by Prof. W. B. Combs and Dr. S. T. Coulter of the Dairy Husbandry Division, both of whom are experienced butter graders. As many of the experimental butters had very strong off-flavors, it was often difficult to score them. The defects were far worse than those encountered in commercial practice. The judges had no knowledge, until afterward, of the nature of the butters under examination, which were scored according to their probable commercial grades.

It is possible for experienced graders to vary by ± ½ grading point for the same sample of butters examined at different times, but once the score of the control butter is decided, the relative values within any one series are fairly exact.

PRELIMINARY BUTTERMAKING EXPERIMENTS

As no test specific for detecting oxidation of the non-fat (including lecithoprotein) fractions of butter was found, experimental butters were made in order to gain indirect information on the relationship between oxidation and flavor deterioration.

In the initial experiments, Nos. 10-16, certain oxidizing agents were added to the creams, and the effects upon the flavor of the butters made therefrom were followed.

EXPERIMENT 10: Comparison of oxidation catalysts: fat-soluble peroxides[3], organic substance (cystein), inorganic substance (ferrous iron). The results are given in Table 8.

The additions of oxidizing substances were obviously overdone. The fat-soluble peroxide caused an extreme amount of oxidation, and even the cystein appears to have had some effect.

EXPERIMENT 11: The effect of adding smaller amounts of fat-soluble peroxide and Fe″ was next tried. The results are given in Table 9. The results for the fat-oxidation tests are not quite consistent with the gradings.

EXPERIMENT 12: The effect of adding still smaller amounts of fat-soluble peroxide and Fe″ was next tried. The results are given in Table 10.

The lowering in score of the samples containing 2 ppm Fe″ (*c* and *d*) after one day's storage is not reflected in the fat-oxidation tests at the end of 64 days' storage.

EXPERIMENT 13: The effect of a mild ripening process was next tried. Cream of initial acidity 0.13 per cent, was held at 50°F. overnight

[3] Benzoyl peroxide, containing 6.6 per cent active oxygen.

Table 8. Effect of Benzoyl Peroxide, Cystein, Fe'' and Cystein plus Fe'' on Flavor and Fat Oxidation in Sweet-Cream Butter

No.	Oxidant	Concentration active oxygen	Grades after keeping at 40°F. for—		Fat-oxidation tests (56 days)		
			1 day	43 days	Peroxide value/ml.	Fat-aldehyde value/ml.	
					Initial	Initial	Heated
a.	Benzoyl peroxide*	200 ppm in fat	Bleached, foreign	Tallowy, foreign, bleached	16.5	11	125‡
b.	Benzoyl peroxide†	200 ppm in fat	Bleached, foreign	Foreign, bleached	23.6	11	125‡
c.	Cystein*	200 ppm in cream	91 Foreign	91 Stale storage	2.3	0.45	0.5
d.	Cystein†	200 ppm in cream	91	89½ Stale storage	1.3	0.55	0.45
e.	Fe''*	10 ppm in cream	Oily, oxidized	89½ Stale storage, metallic	2.2	0.9	1.4
f.	Cystein* Fe''	200 ppm in cream 10 ppm in cream	?Tallowy	89 Stale storage, metallic	2.8	1.0	1.9
g.	Control	.....	92½	90½ Stale storage	1.3	0.4	0.4

* Oxidant added overnight.

† Oxidant added just prior to churning.

‡ Apparently "infinity" with this test.

with 1 per cent of starter culture, and was churned at an acidity of 0.155 per cent. In order to promote oxidation, 2 ppm Fe″ was added to the cream (a) overnight and (b) just prior to churning. The results are given in Table 11.

In these samples of mild starter butter the initial off-flavors promoted by the ferrous iron have actually disappeared during storage.

EXPERIMENT 14: The effect of ripening the cream to a high acidity was next studied. Cream of initial acidity 0.15 per cent was ripened with starter culture overnight to 0.50 per cent acidity. In order to promote oxidation, 2 ppm Fe″ was added to sample *a* overnight. The results are given in Table 12.

The effect of the metal was very small, and the fats were more highly oxidized than usual.

EXPERIMENT 15: Experiments were also done in which traces of Fe″ and Cu″ were added at the salting stage of butter freshly churned from sweet cream. The concentrations of metal added are calculated on the weight of the butter granules and would therefore represent considerably higher amounts in the original creams. The results are given in Table 13.

Even though the metallic contamination is of a rather high order and the storage conditions (72 days at 40°F.) rather drastic, there was apparently no consistent effect on the deterioration which can be ascribed to the metallic catalysts, although the scores at seven days after manufacture are related. The metallic flavors observed at the first gradings disappeared later. This phenomenon—an actual decrease in off-flavor during storage—has been noted in many other cases in the course of this work.

EXPERIMENT 16: In another experiment, butters were churned from sweet cream to which copper was added, one half before pasteurization and the remainder the next day, just prior to churning. The results are given in Table 14.

Again, the addition of relatively large amounts of copper failed to cause any notable flavor defects of the types usually associated with fat oxidation—tallowy, oily, etc.—although there is some relationship between the scores and the proportions of copper added.

These preliminary experiments supported the theory that the presence of traces of oxidizing agents in the cream hastened the deterioration in flavor of mildly salted butters made from pasteurized sweet cream. In addition to oxidized and metallic flavors, woody, stale storage, and stale flavors were observed.

For the remaining principal series of experiments, the metallic catalysts added to the cream (35 per cent fat) were as follows:

Iron, 2 ppm ferrous iron (ferrous ammonium sulphate, freshly made solution)

Copper, 0.1 ppm (as cupric sulphate)

Table 9. Effect of Benzoyl Peroxide and Fe'' on Flavor and Fat Oxidation in Sweet-Cream Butter

No.	Oxidant	Concentration active oxygen	Grades after keeping at 40°F. for—		Fat-oxidation tests (56 days)		
			1 day	49 days	Peroxide value/ml.	Fat-aldehyde value/ml.	
					Initial	Initial	Heated
a.	Benzoyl peroxide*	20 ppm in fat	88 ?Tallowy, nearly bleached	<86 Very tallowy, bleached	5.15	19.2	25
b.	Benzoyl peroxide*	40 ppm in fat	Foreign, quite bleached	<86 Very tallowy, bleached	7.35	10.4	22
c.	Fe''*	5 ppm in cream	88½ Very oxidized	89 Metallic	0.95	0.8	0.9
d.	Fe''†	5 ppm in cream	89 Metallic	90 Slightly metallic, stale	1.10	0.5	3.4
e.	Control	.....	92	90 Stale storage	0.25	0.3	0.4

* Oxidant added overnight.
† Oxidant added just prior to churning.

Table 10. Effect of Benzoyl Peroxide and Fe'' on Flavor and Fat Oxidation in Sweet-Cream Butter

No.	Oxidant	Concentration active oxygen	Grades after keeping at 40°F. for—		Fat-oxidation tests (64 days)		
			1 day	64 days	Peroxide value/ml.	Fat-aldehyde value/ml.	
					Initial	Initial	Heated
a.	Benzoyl peroxide*	10 ppm in fat	91½ Slightly foreign	<86 Tallowy, bleached	1.6	3.95	48
b.	Benzoyl peroxide*	20 ppm in fat	89½ Fairly bleached, oxidized	<86 Tallowy, bleached	2.3	5.0	63
c.	Fe''*	2 ppm in cream	89½ Very stale	89½ Stale storage	0.5	0.25	0.35
d.	Fe''†	2 ppm in cream	90½ Slightly stale storage	90 Stale storage	0.3	0.2	0.3
e.	Control	.....	92	90 Stale storage	0.1	0.25	0.3

* Oxidant added overnight.
† Oxidant added just prior to churning.

Table 11. Effect of Fe'' on Flavor and Fat Oxidation in Sweet-Cream Butter

No.	Oxidant	Concentration Fe''	Grades after keeping at 40°F. for—				Fat oxidation tests (60 days)		
			1 day	8 days	32 days	60 days	Peroxide value/ml.	Fat-aldehyde value/ml.	
							Initial	Initial	Heated
a.	Fe''	2 ppm in cream	89 Slightly metallic	89 Slightly metallic	89½ Stale storage slightly metallic	90 Stale storage	0.3	0.55	.....
b.	Fe''	2 ppm in cream	87 Metallic	86 Very metallic, stale	90½ Stale storage	90 Stale storage	0.4	0.65	.....
c.	Control	.....	92	92	91 Slightly stale storage	91 Slightly stale storage	0.3	0.35	.....

Table 12. Effect of Fe'' on Flavor and Fat Oxidation in Sweet-Cream Butter

No.	Oxidant	Concentration Fe''	Grades after keeping at 40°F. for—				Fat oxidation tests (56 days)		
			1 day	8 days	21 days	56 days	Peroxide value/ml.	Fat-aldehyde value/ml.	
							Initial	Initial	Heated
a.	Fe''	2 ppm in cream	92 Coarse acid	91 Slightly stale and bitter	90½ ? Oily	88 Vinegary, slightly oily	0.85	1.8	2.5
b.	Control	.....	92 Coarse acid	92	91½	89 Slightly oily and stale	1.55	2.1	.....

Table 13. Effect of Cu'' and Fe'' on Flavor and Fat Oxidation of Sweet-Cream Butter

No.	Oxidant	Grade after keeping at 40°F. for—			Fat oxidation tests					
					Peroxide value, 7 days	Fat-aldehyde value/ml.				
		7 days	35 days	72 days		7 days		35 days		72 days
						Initial	Heated	Initial	Heated	Heated
a.	0.05 ppm Cu''	92 Lacks freshness	90 Stale storage	91 Stale storage	0.35	<0.1	0.2	0.15	0.3	0.7
b.	0.1 ppm Cu''	91½ Lacks freshness, bitter	91 Slightly stale storage	91 Stale storage	0.60	<0.1	0.3	0.1	0.6	0.7
c.	0.25 ppm Cu''	91 Slightly metallic	90 Stale storage	90 Stale storage	0.15	<0.1	0.4	0.15	0.5	0.6
d.	0.25 ppm Fe''	91½ Lacks freshness, bitter	90½ Stale storage	90 Stale storage	0.10	<0.1	0.35	0.1	0.6	0.35
e.	0.5 ppm Fe''	90 Metallic, slightly oily	91 Slightly stale storage	91½ Stale storage	0.05	<0.1	0.3	0.15	0.45	0.3
f.	1.0 ppm Fe''	91½ Slightly flat, bitter	90½ Stale storage	90 Slightly metallic and stale storage	0.20	<0.1	0.25	0.15	0.4	0.3
g.	Control	93	91 Slightly stale storage	90½ Stale storage	0.45	<0.1	0.3	<0.1	0.5	0.3

Table 14. Effect of Cu'' on Flavor and Fat Oxidation of Sweet-Cream Butter

No.	Oxidant	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
					1 day		61 days	
		1 day	32 days	61 days	Initial	Heated	Initial	Heated
a.	0.1 ppm Cu''	92 Lacks freshness	91½ Slightly stale storage	88½ Stale storage, oxidized	0.1	0.8	0.35	0.3
b.	0.25 ppm Cu''	91½ Very slightly metallic	92 Slightly stale storage	88 Stale storage, oxidized	0.15	0.6	0.3	0.3
c.	0.5 ppm Cu''	92 Lacks freshness	91½ Slightly stale storage	87 Stale storage, oxidized	0.2	0.6	0.4	0.55
d.	1.0 ppm Cu''	91½ Very slightly metallic	90 Stale storage	88 Stale storage, oxidized	0.15	0.8	0.5	0.95
e.	Control	91 Lacks freshness	92 Slightly stale storage	89 Stale storage, oxidized	0.1	0.5	0.3	0.2

These contaminations are extremely small and appear to be comparable with those found in commercial conditions.

If we assume that the metal is distributed equally among the constituents of the cream, the contamination in the butter would be about 0.8 ppm Fe″ and about 0.04 ppm Cu″. If the metallic salts are present in the buttermilk fractions only, their concentration in the butter would be considerably less. Davies (1933) believes that the metals are preferentially adsorbed on the lecithoprotein "membrane" of the fat globules, which would tend to raise the proportion remaining in the butter. In any case, the average iron and copper contents of commercial butters appear to be about 1 ppm and 0.1 ppm, respectively, and the additions made to the cream in the following experiments are of the order of normal factory contaminations.

It was surprising to find that such a minute trace of ferrous iron had frequently a marked effect on the flavors of the butter. After some time it was noted that ferric iron, added in the same proportion, had no deleterious effect on the flavor, and as probably most of the iron found in butter was originally present in the ferric state, this apparent discrepancy was explained.

Numerous experimental series of butters containing either 2 ppm Fe″ or 0.1 ppm Cu″ in the cream were made and are discussed in Part III. They support the theory that storage staleness and other incipient flavor defects in sweet-cream salted butters are caused by oxidation changes.

II. The Oxidation of Butter Lipides in Relation to Incipient Off-flavors

Most of the results recorded in the previous section show no correlation between the fat oxidation values and the off-flavors. As Henderson and Roadhouse (1934) have already noted, oxidation flavors appear in milk while the fat is still in the very early stages of its oxidation induction period. It would appear that substances other than fat are important in the early stages of oxidation in milk and cream.

Lecithin, or lecithoprotein, has been suggested as being intimately concerned with incipient oxidation changes *(loc. cit.)* in milk and cream, and experiments were accordingly made in order to determine their rôle in the early stages of oxidation in butter.

EXPERIMENTS WITH NATURAL BUTTERFAT EMULSION FREED FROM PLASMA SUBSTANCES BY WASHING

EXPERIMENT 1: Creams consisting of butterfat emulsified in water by the naturally occurring lecithoprotein "membrane" substance were prepared by the washing process described by Palmer and Samuelsson (1924). The washed creams were pasteurized as usual and churned in

glass bottles. In every case the resulting butters were of very rank flavor, metallic and oxidized. The samples churned from unwashed cream (controls) were invariably of good flavor, and approximately 93 score.

It was realized that the washing treatment (at 100-110°F.), requiring about four hours, was rather drastic, and there were other factors, e.g. exposure to diffuse light, aeration in all stages, the presence of approximately 0.1 ppm copper in the distilled water, used in the ratio of 4 or 5 : 1 volume of cream (4 washings), which would encourage oxidation in the cream.

By using a larger stainless steel separator which considerably decreased the time required for the process, and washing with "Seitzwerke-filtered" water acidified to pH 6.7 (copper free), the typical oxidized flavor in butter made from washed cream was not noticeably improved. The results are summarized in Table 1.

Table 1. Butters Churned from Washed Cream (After one day's storage at 40°F.)

No.	Cream	pH Buttermilk	Grading	Fat-aldehyde value/ml.	
				Initial	Heated
a.	Washed	.....	87 Tallowy	.....	2.3
b.	Washed	.....	87½ Metallic	0.3	1.1
	Control	.	93	0.15	0.3
c.	Washed	6.9	89 Metallic and flat	0.2	0.9*
	Control	6.5	93	0.2	0.4
d.	Washed	6.6	<86 Badly oxidized, metallic	0.4	1.5*
	Control	6.6	93	0.1	0.4
e.	Washed	.....	..... Metallic	.....	0.9

* Fat oxidation results obtained after keeping butters 17 days at 40°F.

EXPERIMENT 2: Another series of butters was followed rather more closely. The results are given in Table 2.

This series is interesting as the oxidized flavor of the washed cream butter has improved on keeping, but the fat was definitely more oxidized than the others.

EXPERIMENT 3: As a final attempt to manufacture washed cream butter free from oxidized flavor, cream was washed, taking all of the precautions mentioned, and pasteurized at 160°F. for only 15 instead of 30 minutes. The following results were obtained.

	Grade after 1 day	Fat-aldehyde value/ml. (heated)
Washed cream	90 Slightly metallic	1.4
Control	92	0.5

This was the best butter obtained from washed cream, but the oxidation defects were still present.

Table 2. Butter Flavor and Fat Oxidation of Washed Cream Butters

No.	Cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
		1 day	34 days	63 days	1 day		34 days	63 days
					Initial	Heated	Heated	Heated
a.	Washed	88 Flat oxidized, slightly metallic	89 Very stale storage, slightly metallic	91 Slight storage	0.5	1.35	1.1	1.75
b.	Control	93	92 Slight storage	90 Stale storage	.....	0.4	0.1	0.2
c.	Control +2 ppm Fe''	86 Very metallic	88 Storage, metallic	88 Metallic	.....	0.45	0.25	0.5
d.	Control +0.1 ppm Cu''	92 Slightly flat	91 Stale storage	90½ Stale storage	<0.1	0.4	0.2	0.25

EXPERIMENTS WITH SYNTHETIC CREAMS

As it was found impossible to make butters from washed cream of a quality suitable for work on flavor, experiments were made in order to find whether a synthetic cream could be used.

Actually, this investigation had originally been planned on the assumption that washed or synthetic creams, free from interfering substances in the plasma, could be used as a base for experiments on flavor. It was thought that separate constituents of the plasma, as well as phospholipides, vitamins, etc., could be added before and after treatment with oxidizing agents and the flavor and quality of the resulting butters would then have been directly related to these additions. Unfortunately, it was not found possible to produce butters of good flavor from creams of these types.

A general account of the experiments is probably worth recording, as some of the results may be interesting from other aspects.

PREPARATION OF LECITHOPROTEIN FROM EGGS

The solvents were carefully purified by the following means:

"Hexane": (Skellysolve of boiling range 35-60°C.) treated with H_2SO_4, alkali, etc., and distilled.

Acetone: Treated with moist silver oxide and distilled over $CaCl_2$.

Ether: Kept for several days over charcoal and flake NaOH, and distilled as required.

EXPERIMENT 4: Method A. Fresh egg yolks, usually salted, were repeatedly extracted with "hexane": acetone mixtures, in which the acetone content was decreased until the last extractions were made with "hexane" only (Sell and others, 1935). By carrying out this process in glass bottles, centrifuging to hasten the separations, siphoning off the solvents and drying in a current of nitrogen, finally under diminished pressure at 25-30°C. overnight, the best products were obtained.

Method B. Egg yolks were dried in a vacuum oven at 35-40°C. and extracted repeatedly with cold "hexane," giving a very yellow product.

Method C. Egg yolks were ground to a stiff paste with anhydrous Na_2SO_4 and were extracted with "hexane." The solvent was removed under diminished pressure, and the Na_2SO_4 by dialysis.

Method D. Vacuum-dried egg yolks were extracted with cold "hexane" containing small proportions of ether or acetone, and later by "hexane" alone. The light-colored product appeared to be partly denatured, as it failed to peptize completely in dilute salt solutions.

PREPARATION OF SYNTHETIC CREAMS

EXPERIMENT 5: Synthetic creams were made by emulsifying, at 40-45°C., two volumes of approximately 0.5 per cent lecithoprotein (in 2-4 per cent NaCl solution) with one volume of carefully prepared butter-

fat. The mixture was passed five times through a hand homogenizer, the brass fittings of which were chromium-plated to prevent metallic contamination.

The emulsions, which resembled cream, were fairly stable in appearance and could be washed free from salt by centrifuging in bottles with large volumes of water.

Table 3. Butters Made from Synthetic Creams

No.	Method	Grade after 1 day at 40°F.	Fat-aldehyde value/ml. heated
	0.5% lecithoprotein in 4% NaCl solution		
a.	Washed twice, not pasteurized or salted	Very metallic	1.4
b.	As for *a*	<87 Very metallic	..
c.	As for *a;* old lecithoprotein	Very metallic	4.9
d.	As for *a* with 0.25 ppm Fe'' + trace H_2O_2	Very metallic	12.5
e.	0.5% gelatin used—otherwise as for *a*	Very flat; slightly metallic only	2.8
	Butterfats used for *a–e*	.....	0.3

The synthetic creams churned quite readily, giving butters with strongly oxidized flavors. Results are given in Table 3.

These results suggest that the lecithoprotein is responsible for the development of metallic flavor, which is one of the incipient flavor defects under investigation. Attempts were made to prepare this substance free from oxidation.

EXPERIMENT 6: Attempted preparation of synthetic creams free from oxidation.

Method A was used, and the solvents contained 0.1 per cent hydroquinone as antioxidant. The antioxidant was finally removed either by (a) dialysis or (b) washing on a Büchner funnel with saturated $(NH_4)_2SO_4$ solution. The results are given in Table 4.

Table 4. Butters Made from Synthetic Creams

No.	Method for preparing lecithoprotein	Grade after 1 day at 40°F.	Fat-aldehyde value/ml.	
			Initial	Heated
a.	In presence of hydroquinone and dialysis	86 Very metallic and oily	1.8	11.7
b.	In presence of hydroquinone and washing treatment	<86 Very metallic and oily, quite inedible	1.6	14.6
c.	In presence of hydroquinone which was not removed	88½ Very metallic	1.2	.....
d.	Butterfat used for *a-c*	.....	.....	0.4

EXPERIMENT 7: (a) Lecithoprotein was prepared by the method of Blackwood and Wishart (1934). Egg yolk dispersed in 10 per cent NaCl solution was extracted repeatedly with peroxide-free ether.

Butter made from unpasteurized synthetic cream using this lecithoprotein preparation was 89½ score, slightly metallic, and actually the best product made from synthetic cream.

(b) Another preparation by this method, which was almost white in color, was obtained by diluting the NaCl dispersion of the lecithoprotein greatly with water, washing the precipitate frequently by centrifuging until the odor of ether had almost disappeared. Butters churned from synthetic creams made with this preparation had the properties noted in Table 5. The butters were not metallic but were still unsatisfactory for work on flavors, particularly as the process of pasteurization would doubtless increase the oxidation.

Table 5. Butters Made from Synthetic Creams

No.	Grade after 1 day at 40°F.	Fat-aldehyde value/ml. heated
a and *b*. Butter made from synthetic creams using lecithoprotein prepared as noted in Experiment 7(b)	<87 Oily and insipid	1.5
	<87 Very oily	1.8
c. Butterfat homogenized with dilute NaCl solution, i.e. prepared as *a* and *b* but without lecithoprotein	Foreign, insipid but not oxidized	2.6

The experiments recorded under 5, 6, and 7, as well as other unrecorded attempts to make synthetic butters with desirable flavors using other emulsifying agents, show that emulsions of butterfat are very susceptible to oxidation, irrespective of the emulsifying agent used. In fact, it would appear that the lecithoprotein emulsions—both natural and synthetic—protect the fat, to some exent, from oxidation, although the lecithoprotein itself apparently oxidizes readily to give oxidized and metallic flavors.

This side of the investigation was discontinued.

THE EFFECT OF OXIDATION IN THE FAT ON THE FLAVOR OF BUTTER

Fats obtained from metallic or oily butters prepared from washed or synthetic creams gave high, accelerated oxidation values. Results given so far in Part II suggest that oxidation of the lecithoprotein is responsible for these off-flavors. The fat-aldehyde values indicate oxidation in the triglyceride fraction as well, so the evidence in flavor of the lecithoprotein oxidation is not complete. It was hoped to find further evidence on this problem by following two lines of work: addition of oxidized fats; addition of fat-soluble oxidizing agents to cream used for buttermaking, and subsequently studying the flavor changes and fat oxidation in the products.

EXPERIMENT 8: Addition of oxidized fats to cream.

Butterfat in an extremely oxidized, bleached and tallowy condition was emulsified with 25 per cent cream and churned. The results are given in Table 6.

Table 6. Effect of Tallowy Butterfat on Butter Flavor and Butterfat Oxidation

No.	Type of butter	Grade after 1 day at 40°F.	Fat-aldehyde value/ml.	
			Initial	Heated
a.	Containing 1% very tallowy fat	Very unpleasant, oily, but not typical	0.9	1.35
b.	Containing 4% very tallowy fat	Oily and oxidized, not as bad as *a*	5.4	5.8
c.	Containing 10% very tallowy fat	Metallic, oxidized, almost tallowy	14.0	12.5
d.	Control (emulsified, but no fat added)	Slightly oxidized	0.3	0.3

Butterfat containing 1 per cent of the tallowy fat, i.e. as in *a,* had an extremely rank and tallowy flavor, far worse than any of the butters noted in the foregoing table. The buttermilks also had very tallowy odors, and probably most of the substances responsible for this off-flavor were therefore lost. However, metallic flavors were not found except in *c* which had an initial fat-aldehyde value approximately 100 times that of average butter after removal from storage. The oily flavor is apparently caused by oxidation of the triglycerides. The results support the theory that oxidation of the fat (triglyceride) fraction is not responsible for incipient oxidized flavors of the metallic type. Experiments recorded in Part I and in Part III confirm these observations.

EXPERIMENT 9: The effects upon flavor and fat oxidation of *a,* water-soluble oxidant (Fe″) ; *b,* water-soluble oxidant (Fe″) and water-soluble antioxidant (hydroquinone), and *c,* fat-soluble oxidant (benzoyl peroxide) were compared. Results are given in Table 7.

In *b,* a water-soluble antioxidant (hydroquinone) has prevented the development of metallic flavors in the presence of a water-soluble oxidation catalyst (Fe″). The experiment is not conclusive as hydroquinone is also fat-soluble.

EXPERIMENT 10: This experiment was planned in order to follow the effects of a fat-soluble oxidant, benzoyl peroxide, in the presence of a fat-soluble antioxidant, hydroquinone, both substances being added in small amounts of butterfat which were emulsified with the creams before churning. The creams were pasteurized as usual and held overnight. The results are recorded in Table 8.

The presence of hydroquinone in the fat *c* had no effect on the development of "stale storage" flavor, but when added with a powerful oxidizing agent *b* had a marked effect in retarding oxidized and oily flavor, and as might be expected, repressed the fat-aldehyde value greatly. Sample *b.* at 61 days, scoring 90 yet bleached almost white, is of extraordinary interest.

EXPERIMENT 11: This experiment was a repetition of the previous experiment. The results are given in Table 9.

The results confirm those described in Experiment 10. As is usually

Table 7. Effect of Fe″, Hydroquinone, and Benzoyl Peroxide on Butter Flavor

No.	Additions to cream	Grades after keeping at 40°F. for—			Fat-aldehyde value/ml.				
		2 days	28 days	57 days	2 days	28 days		57 days	
					Heated	Initial	Heated	Initial	Heated
a.	2 ppm Fe″	89 Metallic	90 Slightly metallic	89 Metallic	0.2	<0.1	0.15	.....	0.5
b.	2 ppm Fe″, 100 ppm hydroquinone	92 Very slightly bitter	92 Slightly stale storage	91 Stale storage	0.15	<0.1	0.1	.....	0.2
c.	Fat containing benzoyl peroxide (2.5 ppm active oxygen on fat basis)	..... Solvent	..... Solvent	..... Tallowy	51	0.4	55	2.1	53
d.	Fat, control for c	Solvent	..... Solvent	..... Not tallowy	0.1	0.1	0.15	0.15	0.25
e.	Control	93	92 Slightly stale storage	91½ Stale storage	0.2	<0.1	0.15	.....	0.3

Table 8. Effect of Benzoyl Peroxide and Hydroquinone on Butter Flavor and Fat Oxidation

No.	Additions to cream	Grades after keeping at 40°F. for—			Fat-aldehyde value/ml.		
		1 day	32 days	61 days	1 day	61 days	
					Heated	Initial	Heated
a.	Fat containing benzoyl peroxide, 2.5 ppm active oxygen on fat basis	91 Slightly oxidized	88 Oxidized bleached	88 Oily, very bleached	13.8	0.5	2.4
b.	As for a; and 100 ppm hydroquinone	91 Slightly oxidized	91 Slightly stale storage	90 Stale storage, badly bleached	1.1	0.25	0.4
c.	100 ppm hydroquinone in fat	91 Slightly stale storage	91½ Slightly stale storage	90½ Stale storage	0.5	0.2	0.2
d.	Control—fat only	90 Slightly oxidized	92 Slightly stale storage	90½ Stale storage	0.8	0.2	0.4

the case with butters made from creams to which an emulsion of fat was added, or which were themselves emulsified, the fat-aldehyde values determined on the day after manufacture are high.

It is an interesting fact that butters containing benzoyl peroxide did not develop metallic taints, but the effects of oxidation appeared as stale storage, oxidized, oily and even tallowy flavors. (Part I, Experiments 10-16; Part II, Experiments 9-12.) As the fat-aldehyde values indicated an unusually advanced state of oxidation in these fats, the question arises whether the incipient metallic stage had passed, or whether the oxidation of the triglycerides (and also certain non-saponifiable substances, as shown by the bleaching of the carotene) had been accomplished without the lecithoproteins, which apparently give rise to the metallic flavors on oxidation, being attacked. Experiment 12 was planned to test this point.

EXPERIMENT 12: An emulsion of butterfat containing benzoyl peroxide (equivalent to 2.5 ppm active oxygen on the total butterfat) was added to cream *a,* and to *b* 5 ppm Fe″ was also added. Sample *c* contained an emulsion of butterfat and 5 ppm Fe″, and *d* was a control, containing no added fat. The results are shown in Table 10.

The results suggest that in the presence of fat-soluble peroxide the triglycerides have oxidized independently, eventually giving tallowy flavors.

In the earlier experiments with emulsions of butterfat with lecithoprotein (Part II, Experiments 1-5), metallic taints predominated, but oily and even tallowy flavors also appeared. The fats always showed an increase in oxidation values.

In Experiment 2 (Part II) and other experiments described later in Part III, metallic taints have disappeared on keeping, leaving only stale storage flavors, with an actual increase in score.

Apparently, lecithoprotein, in the metallic stage, upon further oxidation may change to a relatively flavorless substance, with an improvement in quality, or the triglyceride fraction may become sufficiently oxidized to give oily or even tallowy flavors. In Experiments 9-12 (Part II) oxidation has apparently been induced in the triglycerides without affecting the lecithoprotein.

EXPERIMENTS ON THE OXIDATION OF PHOSPHOLIPIDES

An attempt was made to follow the effect of lipide substances on the oxidation of butter. Lecithin, cephalin, and sphingomyelin fractions were obtained from fresh eggs by the method of Bull and Frampton (1936). This process requires only purified organic solvents for the separations and fractionations and dispenses with the rather drastic $CdCl_2$ reagent used by other workers. The operations are carried out at low temperatures, and the process is very tedious. Hydroquinone, as antioxidant, was added to all solvents and removed later.

Table 9. Effect of Benzoyl Peroxide and Hydroquinone on Butter Flavor and Oxidation

No.	Additions to cream	Grades after keeping at 40°F. for—			Fat-aldehyde value/ml.					
		1 day	36 days	57 days	1 day		36 days		57 days	
					Initial	Heated	Initial	Heated	Initial	Heated
a.	Fat containing benzoyl peroxide (2.5 ppm active oxygen in fat)	92 "Not fresh"	89 Very stale storage, bleached	89 Very stale storage bleached	0.6	14	0.55	2.8	0.25	2.1
b.	Duplicate of a	89 Rancid	89 Oxidized, slightly bleached	88½ Very stale storage, bleached	0.55	8	0.85	2.6	0.25	1.6
c.	As for a and 100 ppm hydroquinone	92 "Not fresh"	90 Stale storage, slightly bleached	90 Stale storage, slightly bleached	0.45	1.5	0.45	0.8	0.15	0.5
d.	100 ppm hydroquinone in fat	92 "Not fresh"	91 Slightly stale storage	90½ Slightly stale storage	0.4	0.5	0.5	0.5	0.15	0.35
e.	Control—fat only	92 "Not fresh"	91 Slightly stale storage	90½ Slightly stale storage	0.6	1.0	0.3	0.4	0.1	0.4
f.	Duplicate of e	92½ "Not fresh"	91½ Slightly stale storage	90 Stale storage	0.55	1.3	0.5	0.45	0.1	0.4

Table 10. Effect of Benzoyl Peroxide and Fe'' on Butter Flavor and Fat Oxidation

No.	Additions to cream	Grades after keeping at 40°F. for—			Fat-aldehyde value/ml.					
		1 day	31 days	65 days	1 day		31 days		65 days	
					Initial	Heated	Initial	Heated	Initial	Heated
a.	Fat containing benzoyl peroxide (2.5 ppm active oxygen on fat basis)	89 Stale storage, rancid	..... Tallowy, bleached	86 Slightly tallowy, bleached	0.2	14	1.4	3.4	0.65	1.55
b.	As for a+5 ppm Fe''	88 Metallic	..... Very stale, slightly tallowy	86 Oxidized, bleached	0.2	32	1.1	3.3	1.05	2.2
c.	Fat emulsion, and 5 ppm Fe''	87 Very metallic	90 Very stale	89 Very stale storage	0.1	0.55	0.8	0.8	0.5	0.25
d.	Control—nothing added	92½ Slightly coarse	92 Slightly stale storage	91 Slightly stale storage	0.1	0.5	.....	.....	0.1	0.4

The sphingomyelin and cephalin fractions were further purified by recrystallization from pyridine and ether:alcohol, as described by Levene (1914) and Levene and Rolf (1927), and were finally treated with, and kept under, acetone. The fractions had the following properties.

Lecithin fraction	Cephalin fraction	Sphingomyelin fraction
Waxy solid, almost white	Very faint yellow oil	White powder

Cephalin should be a solid. Apparently through working at too low temperatures, a large proportion of a colorless liquid oil had been precipitated in this fraction. When the cephalin fraction was treated at 70°F., most of the crystals originally present dissolved, and the two fractions obtained had the following properties.

Residue: Waxy substance of low melting point, turning brown and becoming extremely fishy on keeping.

Filtrate: Colorless oil, developing a typical oxidized fat odor on keeping.

The analyses are given in Table 11.

Table 11. Analyses of Phospholipide Fractions

Fraction	Phosphorus*	Total N	Amino N†	Iodine Value (Hanus)	N:P ratio	Theoretical N:P ratio
	per cent	per cent	per cent			
Lecithin	3.70	1.72	0.89	64	1.02	1
Cephalin (residue)	2.14	0.93	0.89	82	0.95	1
Cephalin (filtrate)	0.02	0.02	.....	53	.....	.....
Sphingomyelin	2.42	1.90	0.20	27‡	1.75	2

* Colorimetric comparisons made by Dr. J. W. Nelson, Division of Biochemistry.

† Estimated with ninhydrin against cholamine standard after careful alkaline hydrolysis of phospholipide. Results probably high.

‡ Very poor end point.

Note: The lecithin fraction was undoubtedly a mixture of lecithin and cephalin; the cephalin residue was undoubtedly contaminated mainly with triglycerides which composed the cephalin filtrate; and the sphingomyelin fraction contained some triglycerides and cephalin (also possibly lecithin).

The fractions were emulsified with skim milk and added to cream, the very impure cephalin fraction yielding a poor emulsion.

Unfortunately, it was found later that traces of solvent had remained with the lipides and the flavors were discernible in the butters made with them. The final treatment of the lipides—evaporation of the solvents under diminished pressure—had been as thorough as was considered consistent with avoiding the risk of oxidation, but was obviously insufficient. Only later was it found that these lipide substances retain traces of solvents with unusual tenacity.

EXPERIMENT 13: The effects of adding lecithin, "cephalin," and sphingomyelin fractions to cream used for buttermaking, (1) in the presence of 2 ppm Fe″ as oxidant and (2) with no metal added, were compared. The results are given in Table 12.

Table 12. Effect of Addition of Phospholipides to Cream

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.					
					1 day		31 days		62 days	
		1 day	31 days	62 days	Initial	Heated	Initial	Heated	Initial	Heated
a.	0.21% lecithin+2 ppm Fe"	Foreign—not metallic	Foreign, ?metallic	Solvent, ?metallic	<0.1	0.55	0.1	0.2	0.1	0.2
b.	0.10% sphingomyelin+2 ppm Fe"	Solvent	Solvent	Solvent	<0.1	0.3	<0.1	0.15	0.1	0.3
c.	0.19% cephalin+2 ppm Fe"	Solvent—slightly metallic	Solvent	Solvent, metallic	0.1	0.4	0.1	0.35	0.1	0.1
d.	Control+2 ppm Fe"	93	91 Stale storage	91 Slightly stale storage	<0.1	0.65	0.1	0.2	0.1	0.15
e.	0.21% lecithin	Foreign	Normal storage flavor	Normal storage flavor	<0.1	0.55	0.3	0.35	0.1	0.3
f.	0.10% sphingomyelin	Solvent	Solvent	Solvent	0.1	0.35	0.1	0.2	0.15	0.3
g.	0.19% cephalin	Solvent—no oxidation	Solvent stale	Solvent stale storage	0.1	0.45	<0.1	0.25	0.1	0.3
h.	Control	93	91½ Slightly stale storage	91½ Slightly stale storage	<0.1	0.5	<0.1	0.2	0.1	0.15

Table 13. Effect of Addition of Oxidized Lipides to Cream

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
					1 day		61 days	
		1 day	31 days	61 days	Initial	Heated	Initial	Heated
a.	0.13% oxidized lecithin	89 ?Metallic	89 ?Stale	89 ?Slightly metallic	0.3	0.4	0.15	0.7
b.	0.11% oxidized sphingomyelin	91 Very slightly metallic	91 Slightly stale storage	89 Slightly metallic	0.1	0.35	0.1	0.3
c.	0.16% oxidized cephalin	<86 Extremely fishy	<86 Extremely fishy	<86 Extremely fishy	0.45	1.1	0.9	2.05
d.	Control	92½ Slightly coarse	92 Slightly stale storage	91 Slightly stale storage	<0.1	0.5	0.1	0.4

Despite the somewhat unsatisfactory results due to the presence of minute traces of solvents, there is no evidence that lipides, added in proportions of the order of their contents reported for milk, have any pronounced effect on the oxidation of butter.

EXPERIMENT 14: Portions of the lipide preparations were oxidized by keeping them for 15 hours at 45°C. and for 2 days in diffuse light. They then had the following properties:

Lecithin—brown, odor like ordinary commercial lecithin
Sphingomyelin—white, odorless, apparently unchanged
"Cephalin"—yellow, intensely fishy odor.

These substances were emulsified with skim milk, pasteurized, etc., and churned in the usual way. (See Table 13.)

The metallic flavors noted in Table 13 were not quite typical of this defect. The effect of adding oxidized lecithin is not so great as was expected, and in all probability the slight degrees of off-flavor observed for sphingomyelin were due to impurities, for these fractions were by no means pure. The main interest lies in the "cephalin" sample. The fishy flavor was far more intense than that found in commercial butters with this defect, and this butter actually tasted like low-grade cod-liver oil. It would be interesting to identify the substance—presumably related to the lipides—which gave such a strong off-flavor, for the chemistry of fishiness development in butter is still in dispute. It is generally believed that the breakdown of lecithin to give choline and eventually trimethylamine causes fishiness in butter *(loc. cit.)*, but experiments made early in this investigation failed to agree with this theory.

EXPERIMENT 15: Salted butters were churned from pasteurized creams ripened with starter cultures to low acidity; high acidity, in the presence of choline, traces of Fe″, etc. The results are given in Tables 14 and 15.

None of the samples developed fishiness, although the presence of choline had a marked effect on the development of off-flavors of the metallic and oily types, particularly in the presence of traces of ferrous iron.

EXPERIMENT 16: The effect of lipide substances on the oxidation of butterfat was studied in the following manner.

Butterfat containing 0.3 per cent *(a)* lecithin fraction, *(b)* lecithin fraction (brown, oxidized), *(c)* sphingomyelin fraction was heated for six hours at 80°C. In another series, 0.1 ppm Cu″ (acetate) was added as an oxidation catalyst. The results are given in Table 16.

Lecithin is apparently pro-oxygenic, particularly in the presence of a trace of copper. The mildly pro-oxygenic property of the sphingomyelin fraction, evident only in the presence of copper, may be due to impurities.

Numerous unrecorded experiments were made with artificial creams prepared from old commercial lecithin, brown and oxidized. Oxidation

Table 14. Effect of Addition of Substances Supposed To Cause Fishiness in Butter (Unripened and Mildly Ripened Cream)

No.	Additions to cream	Grade after keeping at 40°F. for—				Fat-oxidation tests (58 days)	
		1 day	15 days	30 days	58 days	Peroxide value/ml.	Fat-aldehyde value/ml.
							Initial
a.*	1% starter+200 ppm choline (overnight)	92 ?Slightly metallic	90½ Stale storage	91 Stale storage	90 Stale storage	1.05	0.5
b.	1% starter+200 ppm choline+2 ppm Fe"	87 Oxidized	88 Metallic	89 Almost tallowy	89½ Slightly metallic	1.15	1.0
c.	1% starter+2 ppm Fe"	90 Stale storage	89 Slightly metallic, oxidized	89½ Oxidized	90 Stale storage	0.30	0.55
d.	1% starter (control)	92	91½ Slightly stale storage	91½ Slightly stale storage	91 Stale storage	0.25	0.45
e.†	200 ppm choline (before churning)	92	91½ Slightly bitter	91 Slightly oxidized	91½ Slightly stale storage	1.35	0.95
f.	200 ppm choline+2 ppm Fe" (before churning)	87 Metallic	86 Very metallic, stale storage	90 Slightly oxidized	90 Stale storage	0.55	0.6
g.	2 ppm Fe"	89 Slightly metallic	88 Stale storage, oxidized	90½ Slightly oxidized	90 Stale storage	0.40	0.65
h.	Control	92	92	91 Stale storage	91 Stale storage	0.30	0.35

* a-d, Initial acidity of cream 0.13%, final 0.155%.
† e-h, No starter added.

Table 15. Effect of Addition of Substances Supposed To Cause Fishiness in Butter (Highly Ripened Cream)

No.	Additions to cream	Grade after keeping at 40°F. for—				Fat-oxidation tests (59 days)		
		1 day	7 days	24 days	56 days	Peroxide value/ml.	Fat-aldehyde value/ml.	
							Initial	Heated
a.	Starter+200 ppm choline (overnight)	92 Coarse acid	91½ Very acid	91 Vinegary	89 Vinegary, slightly oily	2.75	3.6	2.5
b.	Starter+200 ppm choline+2 ppm Fe" (overnight)	89 Metallic	90 Slightly metallic	90 ?Oily	88 Oily	2.35	2.5	3.0
c.	Starter+2 ppm Fe" (overnight)	92 Coarse acid	91 Slightly bitter	90½ ?Oily	88 Vinegary, slightly oily	0.85	1.8	2.5
d.	Starter, control	92 Coarse acid	92 Fair	91½ Fair	89 Stale, oily	1.55	.....	2.1

Table 16. Effect of Phospholipides on Oxidation of Butterfat

No.	Addition to butterfat	Oxidation Tests: Fat-aldehyde values, heated 6 hours 80°C.	Oxidation Tests: Color of fat
a.	0.3% Lecithin fraction (fresh)	0.85	Yellow
b.	0.3% Lecithin fraction (old)	1.0	Yellow
c.	0.3% Sphingomyelin fraction	0.3	Yellow
d.	..... Control	0.3	Yellow
e.	0.3% Lecithin fraction (fresh)+0.1 ppm Cu"	8.8	Bleaching
f.	0.3% Lecithin fraction (old)+0.1 ppm Cu"	8.0	Bleaching
g.	0.3% Sphingomyelin+0.1 ppm Cu"	2.6	Bleaching
h.	..... Control+0.1 ppm Cu"	0.95	Yellow

tests made on the fat in the butters churned from these creams were found to vary, giving anomalous results. This line of investigation was therefore dropped.

Apparently the lecithoprotein, rather than the lecithin, is of importance in the incipient stages of oxidation in dairy products. Other workers *(loc. cit.)* are in agreement with this theory.

III. Investigations on the Antioxygenic Properties of Milk Plasma and the Vitamins

Butters made from washed cream were particularly susceptible to oxidation, as judged both by flavor and fat-oxidation tests. It was noticed at a very early stage of this investigation that butters prepared from washed cream, to which the original skim milk was added after washing, were considerably less oxidized than the samples prepared from washed cream, although never quite so good as the unwashed controls. Table 1 gives results summarized from various series of tests.

Table 1. Antioxygenic Effect of Adding Skim Milk to Washed Creams Used for Buttermaking*

No.	Butter made from	Grade after 1 day at 40°F.	Fat-aldehyde value/ml.: Initial	Fat-aldehyde value/ml.: Heated
Pasteurized 15 minutes at 160°F.				
1.	*a.* Washed cream	90 Slightly metallic	.....	1.4
	b. Washed cream+skim milk†	91½ Very slightly metallic	.....	0.5
Pasteurized 30 minutes at 160°F.				
2.	*a.* Cream	93	0.15	0.3
	b. Washed cream	87½ Very metallic	0.3	1.1
	c. Washed cream+skim milk†	90 Slightly bitter	0.1	0.3
Pasteurized 30 minutes at 160°F.				
3.	*a.* Cream	93	0.2	0.4‡
	b. Washed cream	89 Very metallic and flat	0.2	0.9
	c. Washed cream+skim milk, reseparated	92 Very slightly metallic	0.1	0.3

* See also Table 24 (page 50).

† Containing, in plasma, about 65 per cent skim milk, the remainder being water from the washing treatment.

‡ After 17 days at 40°F.

These results indicated the presence of antioxygenic substances in the milk plasma, and a systematic search for these substances was undertaken.

THE VITAMINS

There is a considerable amount of evidence that vitamin C is antioxygenic in so far as it retards the development of oxidized flavors in market milk (Kende, 1934). Other workers consider that carotene (provitamin A) is the active principle concerned (Anderson and others, 1937; Whitnah and others, 1937).

As the antioxygenic properties of the vitamins in butter have not been studied, so far as can be found, experiments were made, using the plasma-soluble vitamins B, C, and G, as well as the P-P factor (nicotinic acid), and fat-soluble vitamins A, D, and E.

Table 2. Description of Vitamins Employed

Vitamin	Concentration in dairy products	Commercial concentrate used	Amount used*
A	2,120 units/oz. butter (Eddy and Dalldorf, 1937)	Distilled concentrate, 500,000 units A/gram (supplied by General Mills, Inc., Minneapolis)	1.09 gm.
B	7 units/oz. milk (Eddy and Dalldorf, 1937)	"Betaxin" (Winthrop Chem. Co., Inc., New York)	1.05 mgm.
C	14 units/oz. milk (Eddy and Dalldorf, 1937)	Ascorbic acid (Hoffmann-La Roche)	0.369 gm.
D	0.49 units/gram butter (Morgan and Pritchard, 1938)	Crystalline vitamin D in propylene glycol (Winthrop Chem. Co., Inc., New York) 1,000 units/drop	5 drops
E	Known only as equivalence†	Wheat-germ oil (non-saponifiable fraction) (General Mills, Inc., Minneapolis)	0.44 gm.
G	0.12/ml. milk (Whitnah, 1937)	Riboflavin (Hoffmann-La Roche)	1.81 mgm.
P-P Factor	No information—used in same proportions as vitamin C‡	Nicotinic acid hydrochloride (Eastman Kodak Co.)	0.369 gm.

* For "10 times highest" in 5 lbs. 1 oz. 35 per cent cream, yielding approximately 2 lbs. butter.

† It was assumed that butterfat has vitamin E activity equivalent to 125 mgm. wheat-germ oil per 100 gm. butterfat. The product employed represented 5 per cent of a wheat-germ oil having a tested positive vitamin E potency in 500-mgm. dose.

‡ Undoubtedly much too high concentration.

The details of the buttermaking, storage, grading, and fat-oxidation tests have already been described. Traces of metals (2 ppm Fe" and 0.1 ppm Cu") were added to certain of the creams to ensure oxidation, in order that possible antioxygenic effects of the added vitamins might be detected. Control churnings were made with each series.

The amounts of vitamin added were equivalent to (a) 10 times the actual highest amount, and (b) the actual highest amount of the vitamin ever recorded in milk or butterfat. It was assumed that water-soluble vitamins existed only in the plasma, and fat-soluble vitamins only in the butterfat. The vitamins were added to 35 per cent cream, the amounts added being calculated in accordance with the foregoing assumption.

Table 2 gives details of the vitamin preparations used during this series of experiments.

Table 3. Vitamin B

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
		2 days	29 days	60 days	2 days	29 days		60 days
					Heated	Initial	Heated	Heated
a.	B (10 x)	93	91½ Foreign	91 Stale storage	0.35	<0.1	0.25	0.4
b.	Control	92½ Lacks freshness	92 Slightly stale storage	91 Stale storage	0.3	<0.1	0.2	0.5
c.	B (10 x)+2 ppm Fe"	88½ Metallic	88 Metallic	91 Stale storage	0.4	<0.1	0.25	0.4
d.	Control+2 ppm Fe"	89½ Slightly metallic	89 Metallic	91 Stale storage	0.4	<0.1	0.3	0.3
e.	B (10 x)+0.1 ppm Cu"	92½ Slightly bitter	92	90½ Stale storage	0.4	<0.1	0.2	0.4
f.	Control+0.1 ppm Cu"	92 Slightly bitter	90 Stale storage	91 Stale storage	0.35	<0.1	0.35	0.25

Table 4. Vitamin B (Repeated)

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.	
		2 days	34 days	60 days	34 days	60 days
					Heated	Heated
a.	B (10 x)	90 ?Slightly metallic	89½ Very Stale storage	90½ Stale storage	0.1	0.2
b.	Control	93	92 Slightly stale storage	90 Stale storage	0.1	0.2
c.	B (10 x)+2 ppm Fe"	89 Metallic	88 Metallic	89 Metallic	0.1	0.35
d.	Control+2 ppm Fe"	86 Very metallic	88 Metallic	88 Metallic	0.25	0.5
e.	B (10 x)+0.1 ppm Cu"	90 Slightly metallic	90 Stale storage	90 Stale storage	0.15	0.25
f.	Control+0.1 ppm Cu"	92 Slightly flat	91 Stale storage	90½ Stale storage	0.2	0.25

The water-soluble vitamins were added in about 25 milliliters water to the cream (5 lbs. 1 oz., 35 per cent). The metals, also in dilute solution, were added afterward, and the samples were pasteurized after mixing thoroughly.

The addition of the fat-soluble vitamins to cream presented certain difficulties which will be described later.

Water-soluble Vitamins

Vitamin B.—The results as given in Tables 3 to 5 indicated that vitamin B lacks antioxygenic properties.

Table 5. Vitamin B (Equivalent to highest concentration reported)

No.	Addition to washed cream	Grade after keeping at 40°F. for—	
		1 day	32 days
a.	B	92½ Slightly bitter	92 Slightly stale storage
b.	Control	92 Slightly foreign	92 Slightly stale storage

Table 6. Vitamin C

No.	Additions to washed cream	After keeping at 40°F. for 1 day	
		Grade	Fat-aldehyde values/ml. Heated (4 hours only)
a.	C (10 x)	All tallowy 87	1.6
b.	Control		2.3
c.	C (10 x)+2 ppm Fe″		0.8
d.	Control+2 ppm Fe″		3.4

Vitamin C.—Preliminary results, obtained with pasteurized washed cream are given in Table 6. The results indicated antioxygenic properties for vitamin C. Buttermaking experiments were made according to the usual plan. The results are recorded in Tables 7 to 9. Results obtained with butters made from creams containing 10 times the highest reported amounts of this vitamin are interesting. Vitamin C tended to retard the development of oxidized flavors (metallic) in samples containing Fe″ and Cu″, but, in the absence of these metals, the presence of relatively large amounts of this vitamin tended to promote these off-flavors.

Vitamin G.—Preliminary results, obtained with pasteurized washed cream, are given in Table 10. Preliminary results did not indicate antioxygenic properties for vitamin G. Buttermaking experiments, following the usual plan, are reported in Tables 11 to 13. The results indicate an absence of antioxygenic activity for vitamin G.

P-P. factor.—Nicotinic acid was employed for these tests, but the amounts used were probably enormously greater than required. However, this substance appeared to lack antioxygenic properties, as shown in Tables 14 and 15.

Table 7. Vitamin C

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
		1 day	30 days	66 days	Initial	Heated	Initial	Heated
					30 days		66 days	
a.	C (10 x)	93	91 Flat	89½ Metallic, oxidized	0.1	0.5	0.4	0.65
b.	Control	93	92 Slightly stale storage	90½ Slightly stale storage	0.1	0.5	0.3	0.6
c.	C (10 x)+2 ppm Fe"	93	91 Slightly metallic	90 Metallic	0.1	0.5	0.65	0.7
d.	Control+2 ppm Fe"	90 Metallic	87½ Metallic	89 Metallic	0.3	0.8	0.7	0.95
e.	C (10 x)+0.1 ppm Cu"	93	91 Slightly stale storage	91 Slightly stale storage	0.1	0.4	0.4	0.5
f.	Control+0.1 ppm Cu"	93	90½ Slightly oxidized	89½ Stale storage	0.2	0.7	0.55	0.8

Table 8. Vitamin C (Repeated)

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.	
		2 days	35 days	60 days	35 days	60 days
					Heated	Heated
a.	C (10 x)	90 Slightly metallic	90 Stale storage	90 Stale storage	0.25	0.3
b.	Control	93	92 Slightly stale storage	90 Stale storage	0.1	0.2
c.	C (10 x)+2 ppm Fe"	90 Metallic	89 Stale storage	88 Slightly tallowy, metallic	0.15	0.25
d.	Control+2 ppm Fe"	86 Very metallic	88 Stale storage, metallic	88 Metallic	0.25	0.5
e.	C (10 x)+0.1 ppm Cu"	91 Slightly metallic	90 Stale storage	91 Stale storage	0.1	0.25
f.	Control+0.1 ppm Cu"	92 Slightly flat	91 Slightly stale storage	90½ Stale storage	0.2	0.25

Table 9. Vitamin C (Equivalent to highest concentration reported)

No.	Addition to cream	Grade after keeping at 40°F. for—	
		1 day	32 days
a.	C	92 Slightly foreign	91½ Slightly stale storage
b.	Control	92 Slightly foreign	92 Slightly stale storage

Fat-Soluble Vitamins

The effect of adding vitamins A and D, in the presence of traces of Fe″ and Cu″ and also in the absence of added metals, was studied. Suitable control samples were also included.

EXPERIMENT 1: Twenty pounds of butter were carefully "oiled off," and the vitamin A and D concentrates, dissolved in butterfat, were added to portions of this fat. These portions were twice emulsified with buttermilk from pasteurized cream at 500 lbs./sq. inch pressure in the Dairy Husbandry Division's homogenizer. The metals were added as usual, and the synthetic creams were allowed to cool overnight and churned on the following day. When freshly made, the nine butters all had slightly oxidized flavors (90-91 score), and after one month they were all cheesey, indicating bacterial contamination.

It was concluded that pasteurization must be applied to homogenized creams, for the temperatures used, 100-110°F., are very favorable for bacterial growth.

Table 10. Vitamin G

No.	Additions to washed cream	After keeping at 40°F. for 1 day	
		Grade	Fat-aldehyde values/ml. Heated (4 hours only)
a.	G (10 x)	All tallowy 87	2.45
b.	Control		2.3
c.	G (10 x)+2 ppm Fe″		4.1
d.	Control+2 ppm Fe″		3.4

EXPERIMENT 2: Butter was churned from raw cream, and the fat obtained by oiling off at a low temperature. Portions of the fat, containing vitamin A, D, and E concentrates were emulsified with the original raw cream buttermilk. Aqueous solutions of Fe″ and Cu″ were added to some, and the 12 synthetic creams were pasteurized as usual, allowed to cool overnight, and churned. The freshly made butters were all slightly rancid, and after one month scored 90-91 ("stale storage"). Apparently the emulsification treatment had been too severe, causing slight hydrolysis of the fat.

EXPERIMENT 3: To avoid the emulsification process, vitamin A, D, and E concentrates were dissolved in 100 grams of fat, the fat alone being used for the control, and after emulsification with raw cream buttermilk, these synthetic vitamin-rich creams were added to the main

Table 11. Vitamin G

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
					30 days		66 days	
		1 day	30 days	66 days	Initial	Heated	Initial	Heated
a.	G (10 x)	93	90½ Slightly oxidized	90 Slightly stale storage	0.2	0.7	0.35	0.7
b.	Control	93	92 Slightly stale storage	90½ Slightly stale storage	0.1	0.5	0.3	0.6
c.	G (10 x)+2 ppm Fe"	89 Metallic	88 Metallic, oxidized	89 Metallic	0.3	0.7	0.6	0.8
d.	Control+2 ppm Fe"	90 Metallic	87½ Metallic oxidized	89 Metallic	0.3	0.8	0.7	0.95
e.	G (10 x)+0.1 ppm Cu"	93	91 Slightly oxidized	89½ Metallic	0.2	0.7	0.45	0.8
f.	Control+0.1 ppm Cu"	93	90½ Slightly oxidized	89½ Metallic	0.2	0.7	0.55	0.8

Table 12. Vitamin G (Repeated)

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
					2 days	31 days		61 days
		2 days	31 days	61 days	Heated	Initial	Heated	Heated
a.	G (10 x)	92½	91 Slightly stale storage	91 Stale storage	0.35	<0.1	0.2	0.35
b.	Control	92½	92 Slightly stale storage	91 Stale storage	0.3	<0.1	0.2	0.5
c.	G (10 x)+2 ppm Fe"	88 Metallic	88 Metallic, ?tallowy	90 Stale storage, slightly metallic	0.35	<0.1	0.3	0.4
d.	Control+2 ppm Fe"	89½ Slightly metallic	89 Metallic	91 Stale storage	0.4	<0.1	0.3	0.3
e.	G (10 x)+0.1 ppm Cu"	92 Very slightly metallic	91 Slightly stale storage	91½ Stale storage	0.4	<0.1	0.2	0.3
f.	Control+0.1 ppm Cu"	92 Very slightly bitter	90 Stale storage	91 Stale storage	0.35	<0.1	0.35	0.25

Table 13. Vitamin G (Equivalent to highest concentration reported)

No.	Addition to cream	Grade after keeping at 40°F. for—	
		1 day	32 days
a.	G	92 Slightly foreign	92 Slightly stale storage
b.	Control	92 Slightly foreign	92 Slightly stale storage

Table 14. P-P. Factor (Nicotinic Acid)

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.
		1 day	32 days	61 days	61 days
					Heated
a.	P-P. factor (high concentration)	93	92 Slightly stale storage	90½ Stale storage	0.3
b.	Control	93	92 Slightly stale storage	90½ Stale storage	0.2
c.	Control+2 ppm Fe"	89 Metallic	89 Metallic	88 Metallic	0.4
d.	Control+0.1 ppm Cu"	93	91 Stale storage	88 Almost tallowy	0.25

Table 15. P-P. Factor (Nicotinic Acid) (One tenth amount added in previous experiment)

No.	Addition to cream	Grade after keeping at 40°F. for—	
		1 day	32 days
a.	P-P. factor	92 Slightly foreign	91 ?Very slightly metallic
b.	Control	92 Slightly foreign	92 Slightly stale storage

lots of ordinary sweet cream during the usual pasteurization process. The four creams were allowed to cool overnight, divided next day for the addition of metals, and 12 samples churned. The vitamin A and E butters were again unsatisfactory, being fishy.

Before continuing with this work, the vitamin A and E concentrates were purified.

Purification of Vitamin A and E Concentrates

Vitamin A.—The concentrate from fish oil was saponified with carefully prepared alcoholic soda under a current of nitrogen, dissolved in water, cooled rapidly, and shaken out with peroxide-free ether. The solvent was removed in a current of nitrogen and finally under diminished pressure. The thick, brown oil obtained, which was almost free of fishy odor, was dissolved in butterfat and stored for a few days at —5°F. before use.

Vitamin E.—The non-saponifiable fraction from wheat-germ oil, which had a characteristic cereal odor, was purified by the method of Olcott and Mattill (1931). The oily material was dissolved in 92 per

Table 16. Vitamin A

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.		
		1 day	31 days	61 days	1 day	61 days	
					Heated	Initial	Heated
a.	A (10 x) Butter (1)	92 Slightly foreign	90 Stale storage	89 Metallic	0.9	.....	0.6
b.	Control Butter (1)	93	90½ Stale storage	90 Stale storage	0.7	.....	0.25
c.	A (10 x)+2 ppm Fe" Butter (1)	92 Slightly foreign	90 Stale storage	90 Stale storage	1.25	.....	0.75
d.	Control+2 ppm Fe" Butter (1)	89 Metallic	89 Metallic	88 Metallic	0.95	.....	0.4
e.	A (10 x)+0.1 ppm Cu" Butter (1)	92 Slightly foreign	91 Slightly stale storage	89½ Stale storage	0.75	.....	0.7
f.	Control+0.1 ppm Cu" Butter (1)	92 Slightly foreign	91 Stale storage	88 Almost tallowy	1.05	.....	0.25
		1 day	32 days	61 days			
g.	A (10 x)+2 ppm Fe" Butter (2)	90 Foreign	91 Stale storage	91 Stale storage	0.25	0.15	0.95
h.	Control+2 ppm Fe" Butter (2)	91 Stale storage	90½ Stale storage	90½ Stale storage	0.3	<0.1	0.2
i.	Control	93	91 Slightly stale storage	91 Stale storage	0.25	<0.1	0.2

Table 17. Vitamin A (Equivalent to highest concentration reported)

No.	Additions	Grade after keeping at 40°F. for—		
		1 day	35 days	60 days
a.	A Butter (1)	93	92 Slightly stale storage	91 Slightly stale storage
b.	Control Butter (1)	93	91 Stale storage	90 Stale storage
		1 day	30 days	64 days
c.	A Butter (2)	92	92	89 Very stale storage
d.	Control Butter (2)	92	91½ Slightly stale storage	89 Slightly metallic, stale

cent CH_3OH, and extracted with low B. Pt. petroleum ether. The reagents were carefully purified. The petroleum-ether extracts were evaporated rapidly, leaving a brown oil with only a faint odor. The concentrate, dissolved in butterfat, was stored for only a short time at −5°F. before used.

The later experiments were made with these preparations. Nearly every experiment was conducted twice, and the resulting butters designated as (1) and (2), respectively.

Vitamin A.—(See Tables 16 and 17.) The results were rather inclusive, although there was a tendency for the samples containing vitamin A concentrate to be rather better than the others.

Vitamin D.—Tables 18 and 19 give results of the vitamin D tests. The results were inconclusive, although the presence of added vitamin D in normal amounts (Table 19) seems to have retarded the development of storage flavor in butter. However, the vitamin D concentrate used is not necessarily identical in chemical properties with that naturally present in butter.

Vitamin E.—(See Tables 20 and 21.) The results indicated antioxygenic properties for vitamin E for, with a fair degree of consistency, samples containing vitamin E concentrate were rather better than their controls.

The fat-aldehyde values for fats from butters containing added vitamin A, D, and E were erratic at the first examination, which was frequently found in other series, particularly when artificial emulsions of butterfat had been included in the creams. Nevertheless, the final fat-oxidation results, particularly vitamin A and to a less extent vitamin E, indicated pro-oxygenic activity for the vitamins, although the grading results gave evidence of antioxygenic properties for vitamin E and possibly also for vitamin A.

The effect of the purified A and E, and also D, concentrates upon the oxidation rate of butterfat was therefore studied. The vitamins were added at the rate of 10 times the highest reported concentration, the metals as ether solutions of cupric acetate or ferric stearate, and the measurements were made with the standardized accelerated test described in Part I. (See Table 22.)

The results given in Table 22 indicated marked pro-oxygenic activity of vitamin A in the presence of traces of copper and iron. These results

Table 18. Vitamin D

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.		
		2 days	33 days	64 days	2 days	64 days	
					Heated	Initial	Heated
a.	D (10 x) Butter (1)	91 Lacks freshness	91½ Slightly stale storage	90½ Oxidized	0.1	0.15	0.5
b.	Control Butter (1)	92 Lacks freshness	92 Slightly stale storage	90 Oxidized	0.2	0.1	0.5
c.	D (10 x)+2 ppm Fe" Butter (1)	88 Metallic	89 Metallic	88 Metallic	0.15	0.2	0.7
d.	Control+2 ppm Fe" Butter (1)	88 Metallic	89 Metallic	89 Metallic	0.15	0.15	0.55
e.	D (10 x)+0.1 ppm Cu" Butter (1)	91 Lacks freshness	92 Slightly stale storage	90½ Oxidized	0.15	0.15	0.55
f.	Control+0.1 ppm Cu" Butter (1)	91 Stale storage	91½ Slightly stale storage	91 Slightly stale storage	0.2	0.15	0.5
		1 day	32 days	61 days			
g.	D (10 x) Butter (2)	90 Slightly metallic	90 Stale storage	89 Very stale storage	0.25	0.1	0.25
h.	Control+2 ppm Fe" Butter (2)	91 Stale storage	90½ Stale storage	90½ Stale storage	0.3	<0.1	0.2
i.	Control Butter (2)	93	91 Slightly stale storage	91 Stale storage	0.25	<0.1	0.2

Table 19. Vitamin D (Equivalent to highest concentration reported)

No.	Addition to cream	Grade after keeping at 40°F. for—		
		1 day	35 days	60 days
a.	D	93	92 Slightly stale storage	90½ Slightly stale storage
b.	Control	93	91 Slightly stale storage	90 Stale storage

cast some doubt on the value of the experiments recorded in Tables 16 and 17, as it might be reasoned that the vitamin concentrate, despite the precautions taken during its preparation, was already somewhat oxidized when used. On the other hand, the results given in Table 16 *(c-f)* show that, in the presence of traces of copper and iron, added vitamin A concentrate retards the development of oxidation off-flavors, yet raises the values for fat oxidation. Possibly, as vitamin A is a highly unsaturated compound, it has diverted the oxidation from the lecithoprotein, which would normally give a metallic flavor, to the triglycerides, in which the vitamin would be dissolved in the butter.

CHOLESTEROL

Preliminary results obtained with butters containing added cholesterol, either added to the fat used for synthetic creams, or added to washed cream as a colloidal sol, were very erratic, and the work was not continued.

EXPERIMENTS ON THE ANTIOXYGENIC PROPERTIES OF THE CONSTITUENTS OF MILK PLASMA

As recorded in Table 1, Part III, milk plasma has marked antioxygenic properties. As the water-soluble vitamins appeared to be inactive as antioxygens, experiments were undertaken in order to trace the other plasma constituents responsible for this effect.

Butters were made from washed cream containing various plasma substances. The washed cream, 50 per cent fat, was diluted with aqueous solutions or sols to approximately 33 per cent fat content, so that the amount of plasma ingredient added roughly approximated its concentration in cream.

The alcohol-soluble protein was first dialyzed in cellophane tubes and then suspended in water with the aid of a little alkali.

The calcium caseinate and calcium caseinate:calcium phosphate sols[4] were prepared by triturating casein with lime water, etc.

The albumin:globulin fraction was prepared from whey (Palmer, 1926), and the product was finally dialyzed in cellophane tubes.

The creams, containing the plasma substances, were pasteurized in bottles for 30 minutes at 160°F., cooled, churned on the following day, washed but not salted. The results are given in Table 23.

[4] Kindly prepared by Mr. C. L. Hankinson.

Table 20. Vitamin E

No.	Additions to cream	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.		
		1 day	31 days	61 days	1 day	61 days	
					Heated	Initial	Heated
a.	E (10 x) Butter (1)	92½ Slightly foreign	91½ Slightly stale storage	90 Stale storage	1.7	.....	0.7
b.	Control Butter (1)	93	90½ Stale storage	90 Stale storage	0.7	.....	0.25
c.	E (10 x)+2 ppm Fe" Butter (1)	92 Slightly foreign	91 Slightly stale storage	90 Stale storage	0.85	.....	0.35
d.	Control+2 ppm Fe" Butter (1)	89 Metallic	89 Metallic	88 Metallic	0.95	.....	0.4
e.	E (10 x)+0.1 ppm Cu" Butter (1)	92 Slightly foreign	92 Slightly stale storage	89½ Very stale storage	0.85	.....	0.35
f.	Control+0.1 ppm Cu" Butter (1)	92	91 Slightly stale storage	88 Almost tallowy	1.05	.....	0.25
		1 day	32 days	61 days			
g.	E (10 x)+2 ppm Fe" Butter (2)	Solvent	91 Slightly stale storage	91 Slightly stale storage	0.45	0.1	0.55
h.	Control+2 ppm Fe" Butter (2)	91 Stale storage	90½ Slightly stale storage	90½ Slightly stale storage	0.3	<0.1	0.2
i.	Control	93	91 Slightly stale storage	91 Slightly stale storage	0.25	<0.1	0.2

Table 21. Vitamin E (Equivalent to highest concentration reported)

No.	Additions to cream	Grade after keeping at 40°F. for—		
		1 day	35 days	60 days
a.	E Butter (1)	93	91 Slightly stale storage	90½ Slightly stale storage
b.	Control Butter (1)	93	91 Slightly stale storage	90 Stale storage
		1 day	30 days	64 days
c.	E Butter (2)	91 Foreign	92 Slightly stale storage	90 Stale storage
d.	Control Butter (2)	92	91½	89 Slightly metallic, stale

Table 22. Oxidation Rates of Butterfat in the Presence of Fat-Soluble Vitamins and Traces of Metals

No.	Additions to butterfat (1)	Fat-aldehyde value/ml. Heated 6 hours at 80°C.
a.	Vitamin A (10 x)	0.9
b.	Vitamin D (10 x)	1.0
c.	Vitamin E (10 x)	1.8
d.	Control	1.3
e.	Vitamin A (10 x)+0.2 ppm Fe'''	23 (bleaching)
f.	Vitamin D (10 x)+0.2 ppm Fe'''	1.7
g.	Vitamin E (10 x)+0.2 ppm Fe'''	2.4
h.	Control+0.2 ppm Fe'''	2.1
i.	Vitamin A (10 x)+0.1 ppm Cu''	13
j.	Vitamin D (10 x)+0.1 ppm Cu''	5.1
k.	Vitamin E (10 x+0.1 ppm Cu''	3.4
l.	Control+0.1 ppm Cu''	5.6
d'.	Control (unheated)	0.2
m.	Vitamin A (10 x) Butterfat (2)	1.0
n.	Control Butterfat (2)	0.3
o.	Vitamin A (10 x)+0.1 ppm Cu'' Butterfat (2)	1.8
p.	Control+0.1 ppm Cu'' Butterfat (2)	0.95

Table 23. Properties of Washed Creams Containing Various Plasma Ingredients

No.	Additions to washed cream	Concentration in plasma	Grade after 1 day at 40°F.	Fat-aldehyde value/ml.	
				Initial	Heated
		Per cent			
a.	Control (not washed, normal plasma)		93 Normal	0.15	0.3
b.	Control (washed)		87½ Metallic	0.3	1.1
c.	Skim milk (50% normal plasma)		90 Slightly bitter	0.1	0.3
d.	Alcohol-soluble protein	0.1	91 Flat, slightly bitter	0.15	1.45
e.	Calcium caseinate sol (pH 6.6)	3.3	Gluelike	0.2	0.9
f.	Calcium caseinate sol+0.1 per cent colloidal calcium phosphate (pH 6.6)	3.3	Gluelike	0.1	0.65
g.	Albumin : globulin fraction peptized in 13 per cent salt solution	0.4	90½ Slightly metallic	0.15	1.05
h.	13 per cent salt solution (control for *g*)		90 Slightly metallic	0.25	1.55
i.	Lactose	7.5	90 Slightly metallic	0.5	0.8

Table 24. Butters Churned from Dialyzed Creams

No.	Type of cream churned	Grade after keeping at 40°F. for—		Fat-aldehyde value/ml.	
				Initial	Heated
a.	Partly dialyzed only	1 day	*a.* 92 Slightly foreign	<0.1	0.3
			b. 93 Normal	<0.1	0.35
b.	Control	32 days	*a.* 90 Stale, oxidized	<0.1	0.1
			b. 91 Slightly stale storage	<0.1	0.15
		63 days	*a.* 90 Stale storage	<0.1	0.35
			b. 90½ Stale storage	<0.1	0.15
c.	Dialyzed	1 day	*c.* 88 Oxidized, ?metallic	0.5	.....
			d. 90½ Slightly rancid, old cream flavor	0.4	.....
d.	Control	31 days	*c.* 87 ?Metallic	.....	.....
			d. 89 Old cream flavor	.....	.....
		61 days	*c.* 88 Very stale storage, metallic	0.5	1.45
			d. 90 Stale storage	0.5	0.6

As the metal content in commercial samples of lactose is usually high, Experiment 1, Table 23, was repeated, using 5 per cent concentration in the plasma. For this experiment the metals had been extracted from the sugar as diethyldithiocarbamates with ether. The results confirmed the pro-oxygenic activity of lactose as measured by fat-oxidation tests, but, as noted in Table 23, an improvement in flavor was also observed. Apparently the principal plasma-proteins (casein, albumin-globulin), as well as colloidal calcium phosphate, have antioxygenic properties. As these results suggested that the inorganic constituents of milk plasma might be important in this respect, experiments were made with butters churned from creams from which the soluble substances, including inorganic salts, had been removed by dialysis through cellophane.

Several days were required for dialysis, which was judged complete by the absence of a positive Fehling's test. The dialysis water was adjusted to approximately pH 6.6, and was kept at 40°F. The results given in Table 24 confirm the theory that the dialyzable constituents of

Table 25. Butters Churned from Dialyzed and Washed Creams

No.	Type of cream churned	Grade after keeping at 40°F. for—		Fat-aldehyde value/gram	
				Initial	Heated
a.	Dialyzed	1 day	*a.* 87 Foreign, oxidized	.....	.....
			b. 91½ Old cream flavor	.....	.....
b.	Control	16 days	*a.* 89½ Foreign	0.2	0.9
			b. 91½ Stale storage	0.1	0.4
c.	Washed+dialyzed skim milk*	1 day	*c.* 86½ Very metallic	.....	.....
d.	Washed+ordinary skim milk*		*d.* 89 Metallic	.....	.....
			e. <86 Extremely metallic, oxidized	.....	.....
e.	Washed (control for *c* and *d*)	16 days	*c.* 90 Foreign	0.2	0.7
			d. 89½ Foreign	0.1	0.4
			e. 88 Foreign, metallic	0.3	1.1

* Plasma contains 10 per cent added water from washing process.

milk plasma are antioxygenic. In Table 25 results are given of comparisons made between butters churned from *(a)* dialyzed cream and *(b)* control (unheated) cream; and between butters churned from washed cream "reconstituted" with *(c)* dialyzed skim milk and *(d)* ordinary skim milk. The creams were pasteurized, cooled, churned (not washed), and the butters were salted as usual.

Results in Table 25 also indicated antioxygenic properties in both the dialyzable (inorganic salts, etc.) and residual (proteins, colloidal calcium phosphate, etc.) fractions of milk plasma, confirming the results given in Table 23.

EXPERIMENTS ON THE ANTIOXYGENIC PROPERTIES OF INORGANIC SALTS

Certain amino and hydroxy compounds are antioxygens; Lea (1936) has shown that lactates, phosphates, citrates, and others are antioxygenic for lard. Phosphates and citrates occur naturally in milk products. Lactates are found in sour milk, particularly when acidity has been developed by means of a starter culture.

For the following experiments, the sodium salts, in 25 per cent W/V solutions, were used. The solutions were adjusted by means of HCl or NaOH to suitable pH so that on dilution with cream a pH of approximately 6.6 would result. Some difficulty was experienced in achieving this, but the divergences of the pH values of the creams from that aimed at are probably not important. Preliminary results are given in Tables 26 and 27. In order to ensure oxidation, 2 ppm Fe″ was added to the cream after the addition of the antioxygen, just prior to churning. The creams had been pasteurized and cooled as usual, and the butters were salted. The results indicate antioxygenic properties in the compounds studied.

In the next experiment, summarized in Table 28, smaller amounts of antioxygens, including lactates, were tried. The technique was identical with that employed for the previous experiment, but the cream was colder and considerably more viscous than usual, when the solutions of salts were added. The results were not so satisfactory as those obtained from other experiments, probably because the original distribution of these salts was not so thorough as usual. The antioxygenic effects of the added salts were apparent only after the samples had been kept.

For the next experiment, an attempt was made to demonstrate antioxygenic properties for phosphate, citrate, and lactate ions, when added to cream during the process of pasteurization, traces of metals also being added to the creams to promote oxidation. The results are recorded in Table 29.

Results in Table 29 indicate some antioxygenic properties for citrates, and to a less extent, for phosphates, but not after the first month of storage. The fats from the creams with added phosphates were more highly oxidized than the others, and on the two occasions when these

Table 26. Comparison of Antioxygens in Butter

No.	Additions to cream (1)	pH*	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.			
			2 days	28 days	61 days	2 days	28 days		61 days
						Heated	Initial	Heated	Heated
a.	Sodium citrate equivalent to 0.65 per cent citric acid (anhydrous)+2 ppm Fe"	6.9	91 Slightly oxidized	92 Slightly stale storage	91 Slightly stale storage	0.2	<0.1	0.15	0.3
b.	Sodium phosphate equivalent to 0.22 per cent phosphorus+2 ppm Fe"	6.6	93 Normal	92 Slightly stale storage	91 Slightly stale storage	0.2	<0.1	0.15	0.5
c.	100 ppm hydroquinone+2 ppm Fe"	6.4	92 Very slightly bitter	92 Slightly stale storage	91 Slightly stale storage	0.15	<0.1	0.1	0.2
d.	Control+2 ppm Fe"	6.4	89 Metallic	90 Slightly metallic	89 Metallic	0.2	<0.1	0.15	0.5
e.	Control	6.4	93	92 Slightly stale storage	91½ Slightly stale storage	0.2	<0.1	0.15	0.3

* Of "buttermilks" from melted butters.

Table 27. Comparison of Antioxygens in Butter

No.	Additions to cream (2)	pH	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.
			1 day	33 days	63 days	1 day
						Heated
a.	Sodium citrate equivalent to 0.65 per cent citric acid+2 ppm Fe"	6.1	92 Slightly foreign	92 Slightly stale storage	91 Slightly stale storage	0.15
b.	Sodium phosphate equivalent to 0.22 per cent P+2 ppm Fe"	6.55	92 ?Slightly metallic	91½ Slightly stale storage	90 Stale storage	0.15
c.	Sodium lactate equivalent to 0.8 per cent lactic acid	6.4	91 Slightly stale, oxidized	90½ Stale storage	90 Stale storage	0.2
d.	Control+2 ppm Fe"	6.65	90 Slightly metallic	89½ Metallic	88 Metallic	0.15
e.	Control	6.65	93	91 Slightly stale storage	91 Slightly stale storage	0.25

Table 28. Comparison of Antioxygens in Butter

No.	Additions to cream	pH of buttermilk	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.
			1 day	31 days	63 days	Heated 63 days
	Sodium phosphate equivalent to phosphorus—					
a.	0.22% +2 ppm Fe"	6.5	88½ Metallic, slightly oxidized	92 Slightly stale storage	90½ Stale storage	0.85
b.	0.11% +2 ppm Fe"	6.5	90 Slightly stale storage	91½ Slightly stale storage	90 Stale storage	0.6
c.	0.055% +2 ppm Fe"	6.5	90 Slightly metallic	91½ Slightly stale storage	90 Stale storage	0.6
	Sodium citrate equivalent to anhydrous citric acid—					
d.	0.65% +2 ppm Fe"	7.1	91 Slightly stale storage	91½ Slightly stale storage	90½ Stale storage	0.4
e.	0.33% +2 ppm Fe"	7.0	89½ Slightly oxidized	92 Slightly stale storage	89 Very stale storage	0.4
f.	0.16% +2 ppm Fe"	6.9	89½ Metallic	91½ Slightly stale storage	90 Stale storage	0.4
	Sodium lactate equivalent to lactic acid—					
g.	0.8% +2 ppm Fe"	6.7	91 Slightly metallic	91 Stale storage	91 Slightly stale storage	0.45
h.	0.4% +2 ppm Fe"	6.75	90 Slightly oxidized	91 Stale storage	90 Slightly oxidized	0.3
i.	0.2% +2 ppm Fe"	6.65	90 Slightly oxidized	90 Stale storage	89 Slightly metallic	0.4
j.	Control+2 ppm Fe"	6.65	90 Slightly metallic, oxidized	91 Stale storage	89 Slightly metallic and stale storage	0.3
k.	Control	6.65	92	91½ Slightly stale storage	89½ Slightly metallic and stale storage	0.25

Table 29. Butters Made from Cream Heated with Large Amounts of Antioxygenic Salts

No.	Additions to cream	pH buttermilk	Grade after keeping at 40°F. for—			Fat-aldehyde value/ml.
			1 day	32 days	63 days	Heated 63 days
	Sodium phosphate, lactate or citrate equivalent to—		**2 ppm Fe" Added Just Before Churning**			
a.	0.18% P	6.5	92½	92 Slightly stale storage	90½ Stale storage	0.25
b.	0.64% lactic acid	6.75	91 Lacks freshness	91½ Slightly stale storage	89½ Slightly metallic	0.15
c.	0.52% citric acid	6.85	92	92 Slightly stale storage	90 Stale storage	0.1
d.	Control	6.65	91 Lacks freshness	91½ Slightly stale storage	90½ Stale storage	0.1
			0.1 ppm Cu" Added Just Before Churning			
e.	0.18% P	6.5	92	91½ Slightly stale storage	90½ Stale storage	0.6
f.	0.64% lactic acid	6.75	92	91 Slightly stale storage	90½ Stale storage	0.2
g.	0.52% citric acid	6.85	92	91 Slightly stale storage	89½ Stale storage	0.25
h.	Control	6.65	91½	91 Slightly stale storage	90½ Stale storage	0.3
			2 ppm Fe" Added Before Pasteurization			
i.	0.18% P	6.45	90 Almost cheesey	89 Very stale storage	88 Oxidized, stale	0.8
j.	0.64% lactic acid	6.75	91½ Slightly stale storage	90 Slightly metallic	90 Stale storage	0.1
k.	0.52% citric acid	6.85	91½ Slightly stale storage	92 Slightly stale storage	89½ Stale storage	0.3
l.	Control	6.7	91½ Slightly stale storage	91 Slightly stale storage	89½ Stale storage	0.25
m.	Control	6.65	92½	90½ Slightly stale storage	89 Stale storage	0.1
n.	Butter (2) from cream containing 0.1 ppm Cu"	.....	92	91 Slightly stale storage	88½ Oxidized	0.3
o.	As (n)+0.22% P as sodium phosphate	.....	90 Cheesey	92½ Normal	88 Oxidized	0.5
p.	Control	.....	92	92 Slightly stale storage	89 Oxidized	0.2

Table 30. Butters Made from Washed Creams Pasteurized with Phosphates and Citrates

No.	Type of cream churned	pH of buttermilk	Grade after keeping at 40°F. for—		Fat-aldehyde value/ml.		
			1 day	17 days	1 day	17 days	
					Heated	Initial	Heated
a.	Cream (1) (untreated)	6.5	93	93	.....	0.2	0.4
b.	Washed cream	6.9	89 Very metallic, flat	90 Stale storage, slightly metallic	.....	0.2	0.9
c.	Washed cream+skim milk (reseparated)	6.5	92 Slightly metallic	92 Slightly stale storage	.....	0.1	0.3
d.	Washed cream+sodium phosphate in plasma equivalent to 0.22% P	6.65	92½	92 Slightly stale storage	.....	0.15	0.3
e.	Washed cream+sodium phosphate in plasma equivalent to 0.11% P	6.85	91 Slightly metallic	92 Slightly stale storage	.....	0.15	0.3
f.	Washed cream+sodium phosphate in plasma equivalent to 0.055% P	6.8	91 Slightly metallic	92 Slightly stale storage	.....	0.15	0.35
g.	Washed cream+sodium citrate in plasma equivalent to 0.13% citric acid	7.1	92 Foreign	92 Slightly stale storage	.....	0.2	0.3
h.	Washed cream+sodium citrate in plasma equivalent to 0.065% citric acid*	.....	92	92 Slightly stale storage	.....	0.1	0.3
i.	Washed cream*	.....	88 Very metallic, bitter	90 Oxidized	.....	0.15	0.65
j.	Cream (2) (washed)	7.5	.....	.....	0.5	.....	.....
k.	Cream (2) (washed)+skim milk†	6.85	.....	.....	<0.1	.....	.....
l.	Cream (2) (washed)+0.022% P in plasma	7.4	.....	.....	0.2	.....	.....

* Unsalted.

† Plasma contains only 50 per cent of normal plasma solids.

Table 31. Butters Made from Creams Pasteurized at Different pH Values, and in the Presence of Sodium Chloride

No.	Type of cream	pH of buttermilk	Grade after keeping at 40°F. for—		Fat-aldehyde value/ml.	
			1 day	17 days	17 days	
					Initial	Heated
a.	Washed cream	6.8	<86 Badly oxidized, metallic	88 Metallic, foreign	0.3	1.1
b.	Washed cream	6.55	<86 Very metallic, oxidized	88 Metallic, foreign	0.4	1.5
c.	Washed cream	5.65	<86 Intensely metallic	86 Very metallic, foreign	0.75	3.1
d.	Washed cream	4.1	<86 Metallic, more than *c*	<86 Extremely oily	2.0	20.8
e.	Washed cream	6.95	88 Metallic	88 Metallic, foreign	0.3	1.0
f.	Washed cream	7.4	89 Slightly metallic	89 Foreign	0.35	0.95
g.	Washed cream	8.75	91 Very slightly oxidized	90 Foreign	0.25	0.9
h.	Washed cream containing 11% NaCl in plasma	5.85	<86 Intensely metallic	86 Intensely metallic	0.8	3.8
i.	Washed cream containing 11% NaCl in plasma	6.45	87 Metallic	88 Foreign	0.4	1.5
j.	Washed cream containing 11% NaCl in plasma	7.2	89 Slightly metallic	88 Foreign	0.5	2.2

creams were pasteurized in the presence of traces of metals *(i)* and *(o)*, the butters were cheesey when first made, indicating, it is believed, degradation of the plasma proteins.

Apparently, the conditions for this experiment were altogether too drastic, the addition of relatively enormous concentrations of salts and the heat treatment during pasteurization (30 minutes at 160°F.) causing secondary changes which overshadowed the antioxygenic effects expected.

Unfortunately, there was no time to repeat this experiment, using smaller concentrations of salts, but experiments recorded later show that with smaller amounts of phosphates and citrates, beneficial results are obtained with butters churned from washed creams. For these experiments, washed creams were pasteurized with different amounts of phosphate and citrate, and the salted butters were scored for flavor and examined for fat oxidation. The results are given in Table 30.

Finally, the effects of hydrogen-ion concentration and the presence of a neutral salt (NaCl), added to washed cream before pasteurization, were followed. The hydrogen-ion concentration was varied by means of small amounts of $\frac{N}{10}$ HCl or NaOH solutions. The creams containing the salt (11 per cent NaCl in the plasma, approximating the concentration in the buttermilk fraction of commercial salted butter) were also adjusted to different pH values. The sodium chloride (C.P.) was freed from traces of copper and iron by extracting the metals as diethyldithiocarbamates with ether. The butters churned from the unsalted creams were brought to approximately the same salt concentration of the others by working, in the usual way, after churning. The results are given in Table 31.

The results given in Table 31 indicate the effects of different hydrogen-ion concentrations and of metal-free salt on butters churned from creams reduced to their very simplest composition—fat, lecithoprotein "membrane" substance, and water. As the pH value of the creams was lowered, the flavor of the butters became increasingly metallic and the fat oxidation values rose sharply.

When the pH value of the creams was higher than usual *(e-g)*, the grades of the butters improved. In fact, these three samples were definitely superior to those of pH values 6.8 and 6.55 *(a* and *b)*, which are normal for sweet-cream butters. The fat-oxidation values were unaffected by the higher pH values.

Possibly there were traces of catalytically active metals adsorbed on the lecithoprotein, which were ionized under acid conditions, promoting the oxidations recorded *(b-d)*, but under alkaline conditions *(e-g)* the metals would be precipitated, probably as hydroxides, and would therefore be inactive. Possibly the antioxygenic activities of the phosphate and citrate ions can be explained by this simple theory.

Surprisingly enough, the presence of metal-free salt, added before pasteurization, had no notable effect on either the flavor or fat-oxidation

values (cf. *b* and *i; c* and *h; f* and *j*). The results obtained in Part I in which salted butters gave evidence of more fat oxidation after storage than the unsalted products were probably due to the presence of traces of metallic oxidation catalysts (Fe″ and Cu″) added with the salt.

Summary

In butters of low salt content, churned from pasteurized sweet cream, incipient off-flavors such as flat, bitter, stale storage, and metallic may be promoted by oxidation catalysts, including traces of copper and ferrous iron. Ferric iron is inactive.

These incipient oxidation changes may occur long before there is any evidence of oxidation in the butterfat. Moreover, the addition of oxidized fats, or of fat-soluble peroxides dissolved in fat, to butter fails to produce off-flavors of the types referred to, but tends to promote oxidized, oily or tallowy flavors which are, as in other fats, due to oxidation of the unsaturated fat acids in the triglycerides.

It would appear that oxidation of the lecithoprotein (present in the cream as the natural emulsifying "membrane" substance surrounding the fat globules) is responsible for the incipient off-flavors, of which the most objectionable is the metallic type.

The oxidation of the lecithoprotein "membrane" material in cream is evidently transmitted to the triglycerides of the fat globule, for in most cases the oxidation is eventually reflected by an increase in the fat-oxidation (fat-aldehyde) values.

Experiments made with carefully prepared butterfat showed that 0.1 ppm Fe‴ or 0.01 ppm Cu″ are effective as oxidation catalysts. Fats obtained by carefully melting butters recently churned from creams containing traces of Fe″ and Cu″ usually gave no significant increase in fat-oxidation values, which supports the view that the effect of the metals is an indirect one, acting through the lecithoprotein. The metals are preferentially adsorbed on the lecithoprotein "membrane" *(loc. cit.)*.

The addition of relatively large proportions of Cu″ or Fe″ to the butter granules at the working stage, i.e. after the O/W emulsion originally present in the cream has been destroyed, had but little effect on the flavor of the butter even after storage.

Milk or cream appears to be susceptible to oxidation because the minute fat globules, surrounded by the lecithoprotein "membrane" material, present an enormous surface to the dissolved air in the plasma. However, it is not presumed that the lecithoprotein is pro-oxygenic; in fact, some preliminary experiments have indicated that it is probably otherwise. The lecithoprotein "membrane" appears to be more readily oxidized in the presence of catalysts, or in the absence of certain natural antioxygenic substances.

Experiments were made to find whether other non-fat ingredients of butter were oxidizable, but it appeared that the constituents of buttermilk were rather resistant toward oxidation. No evidence of oxidized

proteins could be found in commercial butters with various types of off-flavors.

Later, it was found that the plasma substances are highly antioxygenic. Butter made from washed creams, or synthetic creams prepared from butterfat emulsified with egg-lecithoprotein, were invariably of rank, metallic or oxidized flavor, with relatively high fat-oxidation values. Washed creams reconstituted with skim milk before pasteurizing gave butters showing no more fat-oxidation than in the control (unwashed churning) but with very slight off-flavors, due to changes in the lecithoprotein during the short washing treatment, which could not be avoided.

Butters made from dialyzed creams have more highly oxidized fats than those made from the untreated (control) creams, but are less oxidized than butters made from washed creams. The gradings for flavor support these findings.

There are actually antioxygenic substances in the non-dialyzable, colloidal constituents of milk plasma. This observation was confirmed by other tests.

Much of the antioxygenic effect of milk plasma would appear to be due to the presence of soluble phosphates and citrates. These are known to be antioxygenic for other fats *(loc. cit.)* and have proved also to be effective antioxygens in cream used for buttermaking. Even when used alone in washed creams, in amounts in the range of their reported concentrations in the plasma — soluble phosphates, approximately 0.05 per cent (Lampitt, Bushill and Filmer, 1937); citrates, approximately 0.1 per cent (Supplee and Bellis, 1921) — their effects were surprising.

Off-flavors of the metallic type have frequently improved during storage, leading to an actual increase in the score of the butter. Apparently oxidation of the lecithoprotein may reach a stage where, on further oxidation, the defective flavor is lost. Rogers (1937) has made a similar suggestion in connection with studies on the so-called oxidized flavor of milk.

When metallic and other rank off-flavors decrease in intensity, the flavor of the butter never approaches its original freshness, but is of the stale-storage type.

A certain amount of stale-storage flavor seems to be almost inevitable in butter when kept, and, as noted elsewhere, it appears to be an oxidation defect. It may be found in all types of experimental butters, even those churned from washed creams. Presumably it is caused by incipient changes in the fat, but these are so slight that it has not been found possible to detect them by chemical analysis.

Most of the present-day investigations on incipient oxidation flavors in milk products are concerned with the possible antioxygenic properties of the vitamins, lipides, and carotene.

Experiments were made early in this work with butters made from pasteurized creams containing relatively large concentrations of added vitamins A, B, C, D, E, G, and "P-P. factor" (nicotinic acid), but of

these only vitamins C and E appeared to have antioxygenic properties. Most of the current research centers around vitamin C, which in this investigation was not found to be consistently antioxygenic even in high concentrations. The results obtained with the other plasma ingredients, particularly the inorganic salts, were much more striking.

Discussion and Conclusions

From a biochemical standpoint, it is considered that the oxidation changes responsible for deterioration in sweet-cream, salted butter probably occurs in accordance with the following theory.

Cream is an oil/water emulsion containing butterfat emulsified by means of the naturally occurring lecithoprotein "membrane" substance. According to the modern theories of emulsification, the lipide portion of the lecithoprotein complex is in the fat globule, and the protein portion (globulin-like, but containing a prosthetic group[5]) extends into the aqueous phase of plasma.

Metallic contaminants are adsorbed on the proteins, but preferentially on the lecithoprotein membrane substance (Davis, *loc. cit.)*.

In the presence of dissolved air—or possibly even by a process of anaerobic oxidation—the minute traces of adsorbed metals, particularly in the process of high-temperature pasteurization, catalyze the oxidation of the lecithoprotein, which tends to transmit the oxidation to the triglycerides in the fat globules. The process is one of heterogeneous chain catalysis.

However, there are also present antioxygenic substances—proteins with $-NH_2$, $-OH$, and $-PO_4$ groups, which, though colloidal and therefore not dissolved in the plasma, exert an antioxygenic effect through the action of these polar groups. Also in the aqueous phase of the cream (plasma), there are dissolved phosphates, citrates, vitamin C, and possibly other substances which are antioxygenic. The combined effect of these antioxygenic substances, whether present as solutes or colloids, may be regarded as one of negative heterogeneous catalysis.

The exact nature of the initial changes are bound up with the oxidation-reduction potential of the system, which, as can be seen, is very complex and little understood.

However, a stage may be reached in the cream when the antioxygenic properties are overcome by the oxidation effects, and the lecithoprotein becomes oxidized, which will tend to lead to the development of off-flavors later in the butter.

The incipient oxidation off-flavors usually appear, as has been shown, as flat, bitter, metallic, and stale storage.

The process of churning cream into butter completely changes the colloidal nature of the product. According to modern theories of churning, the cream, which is an oil/water emulsion stabilized mainly by the lecithoprotein "membrane," is inverted to what is essentially a water/oil

[5] Palmer and Samuelsson (1924); Palmer and Wiese (1933).

emulsion by the churning and working processes. The emulsion is destabilized by the violent mechanical process of churning, the lecithoprotein "membrane" being worn away, so to speak, by the effects of concussion and attrition, until the emulsion breaks and the fat globules collect as granules. Most of the plasma substances, as well as a considerable portion of the lecithoprotein, are rejected with the buttermilk. The working of the butter completes the inversion by actually kneading the fat (triglycerides) into a continuous phase in which the buttermilk, containing plasma proteins, lactose, salts, as well as lecithoprotein (and added salt) are distributed.

In the case of butter churned from pasteurized sweet cream, particularly when salted, we have no reason to believe that the conditions in this product could be other than tending to promote fat oxidation, particularly as 4 to 6 per cent by volume of air is naturally present.

Any incipient oxidation of the lecithoprotein or triglycerides brought about during the pre-churning stages of the product will also be present in these fractions in the butter, and on further oxidation may advance in various ways.

Usually, butter of average quality will be free from flavor defects, and during prolonged storage will develop only "storage staleness"—often discernible only by a trained butter-grader—which probably comes from incipient oxidation in the triglyceride fraction and is undetectable by chemical analysis.

However, the stage of oxidation may initially be high enough to impart bitter or metallic flavors, or the initial oxidation in the lecithoprotein may increase during storage and give these defects. If these types of off-flavor are initially present, they may on further oxidation either increase to a maximum or disappear, giving an actual improvement in the flavor of the product.

Incipient oxidation of the lecithoprotein, when it is intimately associated with the fat globules of the milk or cream, also affects the triglycerides, it is believed, although these changes cannot be detected by relatively crude methods of chemical analysis available, as the fat is still in the very early stages of its oxidation-induction period. After churning, the fat will continue to oxidize and may reach a stage where defects undoubtedly arising from oxidation of the unsaturated fat acids appear, e.g. oxidized (probably), and oily and tallowy flavors. With ordinary, well-made butter this is now quite uncommon. In all probability the oxidation will have advanced, by the time the butter is consumed, only as far as to promote a marked "stale-storage" or oxidized flavor.

Other off-flavors, from the lecithoprotein fraction, may also be present, and the combination of off-flavors from this and the triglycerides may impart a variety of flavor defects which are difficult to classify, particularly in view of the fact that the off-flavors in the lecithoprotein fraction may actually improve during storage. There are, therefore, at least two concurrent processes of oxidation concerned in the deterioration of butter.

This theory accords well with numerous observations noted in this investigation where the effect of adding traces of Fe″ to cream before pasteurization has caused rather pronounced off-flavors in the butters when freshly made, but after one or two months' further storage at 40°F., the defects have eventually appeared only as a stronger "storage staleness" than present in the control samples.

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